SCIENTIFIC PROCEEDINGS.

VOL. XXV. OCTOBER, 1927. No. 1.

New York Meeting.

Post Graduate Hospital, October 19, 1927.

3666

The Composite Nature of Vitamin B.

ROBERT R. WILLIAMS AND ROBERT E. WATERMAN.

From the Department of Physiological Chemistry, Teachers College, Columbia University.

Chick and Roscoe[1] have recently reviewed and amplified the evidence that vitamin B involves two factors of importance for young rats, one thermolabile, the other stable under several hours autoclaving at 125°. Seidell[2] has shown that the stable factor does not noticeably contribute to the well-being of pigeons on a polished rice diet. In this paper we present evidence confirming these findings and throwing some further light on the multiple nature of vitamin B.

We used as supplements (1) aqueous extract of brewers yeast, (2) fullers earth activated with the same extract with interposition of a collodion membrane, as described in a forthcoming paper in the *Journal of Biological Chemistry,* and (3) brewers yeast which had been autoclaved for 6 hours at 125°. As a basal diet we used a typical synthetic preparation, the No. 107 diet of Sherman and Spohn.[3] In all cases the diets were fed *ad libitum,* and the supplements separately, so as to insure their complete consumption. Identical lots of material were used throughout the experiments tabulated.

We have been led to conclude: (1) That the dietary requirements of adult pigeons are distinctly different from those of young rats, since identical diets gave contrasting results, *cf.*, Exp. 5 and 6. (2) That young rats require two B factors, one of which is of little or no consequence to pigeons. (3) That, on the other hand, pigeons require something not required by young rats, since normal weight of pigeons was not maintained on a diet that supported normal growth in rats. See Exp. 6. This third unknown factor required by pigeons but not by rats is present in whole wheat, for birds assume a higher and normal level of weight when fed whole wheat.

TABLE OF EXPERIMENTS

With Synthetic Diet of Sherman and Spohn

Exp. No.	Animal	Supplement	Daily Dose Grams	Result
1	Pigeon	Yeast extract	.2–.4	Decline to about 80–90% of normal weight. Healthy except for slight dejection of attitude.
2	Rat	Yeast extract Autoclaved yeast	.03 1.0	Normal growth.
3	Rat	Activated fullers earth	.01	Scanty growth followed by slow decline of several weeks duration.
4	Pigeon	Ditto	.01	Same as Exp. 1.
5	Rat	Activated fullers earth Autoclaved yeast	.01 1.0	Normal growth.
6	Pigeon	Ditto	"	Same as Exp. 1.
7	Rat	Autoclaved yeast	1.0	Scanty growth, followed by decline, polyneuritis and death.
8	Rat	None		Rapid decline, polyneuritis and death.
9	Pigeon	"		Decline in weight to 60 to 70% of normal, followed by polyneuritis and death in 30–40 days.

The same experiments have been performed with pigeons, substituting polished rice for the synthetic diet. The results were essentially the same except that the birds receiving no supplement died sooner. We believe this is evidence that our synthetic diet was not quite free from the thermolabile B factor, and suspect this defect has often crept into the work of others, since complete removal by extraction processes is extremely difficult, if not impossible. However, the absence of the third unknown factor in adequate amount in polished rice is clear. The failure to maintain weight is not due to a partial deficiency of the thermolabile antineuritic factor, for the use of 8 times as large doses of the activated fullers earth did not bring the weight up to normal.

This third factor is evidently not vitamin A or D, which were abundantly present in the synthetic diet in the form of butter and cod liver oil. It is improbable that it is an ash constituent, in view of the salt mixture in the synthetic diet. It may be of the nature of vitamin E, since no very rich source of E was present in abundance. We are endeavoring to ascertain the nature of this third factor.

---

[1] Chick, H., and Roscoe, M. H., *Biochem. J.*, 1927, xxi, 698.

[2] Seidell, A., *Bull. Soc. Chim. Biol.*, 1926, viii, 746.

[3] Sherman, H. C., and Spohn, A., *J. Am. Chem. Soc.*, 1923, xlv, 2720.

3667

Effect of Thyroparathyroidectomy on the Jaundiced Animal.*

WILLIAM C. BUCHBINDER AND RUTH KERN. (Introduced by O. T. Schultz.)

From the Nelson Morris Institute for Medical Research of the Michael Reese Hospital, and the Department of Physiology of the University of Chicago.

During the course of some experiments on jaundiced puppies we noted a fairly progressive lowering of the blood serum calcium, which, in several instances fell to the tetany level.[1, 2] None of the animals evinced the slightest evidence of increased neuromuscular excitability; on the other hand, some degree of apathy was the rule. It was thought that either some of the circulating biliary constituents or some one or more intermediary products resulting from the perversion of liver function had raised the threshold of nervous excitability, or, that the parathyroids had assumed an added function and were playing some rôle in keeping the animals out of tetany. Apart from such intercepting factors there was every reason to expect the early precipitation of tetany in very young animals with extensive morphologic changes in the liver, a markedly disturbed intestinal condition and in addition a lowering of the blood serum calcium. To remove suspicion from the parathyroids, a series of animals, young and adult, were, after a period of jaundice lasting 16 to 18 days, induced by division of the common duct between ligatures, subjected to thyroparathyroidectomy. Blood calcium determinations were made in the normal, jaundiced and parathyroprivic states.

In the first group of experiments puppies, 8 to 10 weeks old, were used. Only slight symptoms of tetany were noted and in more than

---

* Work done under the Nelson Morris Fund and the John D. and Fannie K. Hertz Fund of the Michael Reese Hospital.

TABLE I.
Animal No. 3 (C. J. S.), Mongrel.

Date	Time	Blood calcium	Food	Remarks	Weight
		mgm.			lbs.
May 6	9:30 a. m.	11.9	Stock diet	Division of common duct	25½
May 24	9:00 a. m.	11.7		Thyroparathyroidectomy	22
May 25	9:00 a. m.	7.4	150 g. meat	Occasional flapping of ears	
	12:00 m.			Slight excitability	
	5:00 p. m.			Quiet	
May 26	8:30 a. m.		Milk, 6 oz.	Snapping of jaws while drinking	
			Cereal, 8 oz.	Gait unsteady	
	4:00 p. m.			Gait ataxic	
May 27	8:00 a. m.	7.4	250 g. meat	No tetany	
	4:00 p. m.			Slight fibrillation of scalp muscles	
May 28	8:00 a. m.			No tetany. Refuses food	
	5:00 p. m.			Quiet	
May 29	8:30 p. m.		250 g. meat	Slight fibrillation of scalp muscles	
	4:00 p. m.		100 g. bread	No tetany	
May 30	8:30 a. m.		300 g. meat	Some tremors noted	
	12:00 m.			No tetany	
	5:00 p. m.			No tetany. Marked weakness	
May 31	8:30 a. m.	4.9	Cereal, 8 oz.	Very slight tetany after drawing blood	
	2:30 p. m.		Milk, 6 oz.	Slight tetany	
	4:30 p. m.			Very slight tetany	
June 1	8:00 a. m.		150 g. meat	Marked weakness and depression. No tetany	
	12:00 m.		100 g. meat	Evinces jealousy of other animals	
	5:00 p. m.			No tetany. Bloody stools	
June 2	8:30 a. m.		None	Flapping of ears. No tetany	
				Barely able to walk about	20
	5:00 p. m.			Slight fibrillation of scalp muscles	
June 3	8:30 a. m.	3.6	200 g. meat	Very cachectic	
				Slight fibrillation of scalp muscles	
	4:00 p. m.			No tetany. Brady cardia with a tendency to bigeminus	
June 4	8:30 a. m.		250 g. meat	Fibrillation as above	
	4:30 p. m.			No tetany. Heart rate regular	
June 5	9:00 a. m.		150 g. meat	Slight fibrillation	
	12:00 m.		100 g. bread	Bloody stools	
	4:00 p. m.			Incipient tetany when disturbed	
June 6	9:00 a. m.	3.6		No tetany. Profound cachexia	17½
	12:00 m.		None	Refuses food and water	
	5:30 p. m.			On attempting to walk shivering of extremities and ataxia	
June 7	8:00 a. m.			Found dead. Legs not outstretched	

half of the animals manifest tetany did not occur. The symptoms were not altogether unlike those described by Blumenstock and Ickstadt[3] for the Eck-fistula parathyroidectomized animal, and resembled an acute intoxication, the animal usually becoming moribund before death and dying without tetany. The survival period was considerably longer than their nonjaundiced litter mates which developed a most severe tetany. In most instances the blood serum calcium fell to very low levels within 24 hours after parathyroidectomy. The feeding of meat had no effect on the time of appearance or the severity of the tetany, nor, did it seem to influence the survival period, as compared with those that were deprived of the meat.

In the group of adult animals, parathyroidectomized after a period of jaundice of equal duration, severe tetany ensued in a few animals, but, in the greater number the tetany was markedly diminished in severity. The survival period for 5 out of 7 animals was in each instance over 7 days. One animal lived 17½ days. There was a gradual decrease in calcium values to 3-5 mg., before the ninth day. These animals all received stock diet. The typical course of such an animal is shown in Table I.

Conclusions. There is a marked amelioration in the tetany symptoms of parathyroidectomized animals jaundiced for some time previously. Their survival period is longer. There is probably a raising of the threshold of nervous excitability by one or more of the biliary constituents or products arising from a perversion of liver function. The evidence is against any rôle of the parathyroids in jaundice.

---

[1] Buchbinder, W. C., and Kern, R., *Am. J. Physiol.*, 1927, lxxx, 273.

[2] Buchbinder, W. C., and Kern, R., Growth Factor in Defective Calcification and Blood Calcium Deficiency in Experimental Obstructive Jaundice. In press.

[3] Blumenstock, J., and Ickstadt, A., *J. Biol. Chem.*, 1924, lxi, 91.

3668

Mechanism of Cataract Formation in Thyroparathyroidectomized Dogs.

CHARLES EISEMAN AND ARNO B. LUCKHARDT.

From the Department of Physiology of the University of Chicago.

This work was suggested by the reported occurrence of bilateral cataracts in old thyroparathyroidectomized dogs.[1] Symptoms of parathyroid deficiency were simulated in normal dogs while observing the lenses of these dogs by oblique illumination, and, by opthalmoscopy at a distance, with the aid of atropine. The symptoms of tetany were simulated. These were thought by clinicians of the latter half of the last century to produce cataracts,[2] decreased blood calcium,[3] increased blood phosphate,[4] and an attempted increase in the deposition of calcium.[5]

Tetany was induced by the intravenous injection of oil of wormwood, and the subcutaneous injection of strychnine sulphate. In 18 injections, by the first method, we produced some 49 tonic seizures, and a total of 1 hour and 38 minutes of clonic seizure. The two dogs which lived through this treatment were under observation for 3 months. The dogs receiving strychnine, 20 injections resulting in 26 convulsions, were held 2½ months for observation. Neither method yielded us a single cataract.

We attempted to lower blood calcium by intravenous injection of solutions of sodium oxalate at the level of 30 mg. per kilo, and also $Na_2H\ PO_4$ solutions, in doses varying from 0.386 gm. to 0.690 gm. per kilo. The oxalate was given in 1 dose, while the Na_2HPO_4 was given in 3 daily portions. The animals lived only 6 days, but, during this time we could discover no indication of cataract formation.

To increase blood phosphate we administered NaH_2PO_4 solution *per os* at levels from 3.6 to 5.8 gm. per kilo, given as 3 daily portions for 16 days. The Na_2HPO_4 dogs cited above also had received phosphate. We could detect no cataractous change in the lenses as a result of this treatment.

In attempting to increase calcium deposition we reasoned that there must be an equilibrium between the various tissues, including the lens, and that if we added soluble calcium, or increased phosphate, or alkalinized the blood, or combined these methods, we might succeed in depositing calcium, that is, in producing cataracts. These ideas were tested by the intravenous injection of calcium lactate at levels from 0.04 to 0.06 gm. per kilo; by the intravenous injection of calcium lactate at the level of 0.1 gm. per kilo, and, giving 0.75 gm. of NaH CO_3 *per os;* by the intravenous injection of calcium lactate at a level of 0.09 gm. per kilo, and by administering *per os* NaH_2PO_4 at 0.275 gm. per kilo; by interchanging the routes of the last procedure, giving 0.07 gm. of calcium lactate *per os,* with intravenous injection of NaH_2PO_4 at a level of 0.27 gm. per kilo. These last two groups of dogs lived only one week; the others received treatment, in some cases, for 22 days, and were observed for the following 7 months. No cataracts were seen.

We have negative results only, except that, contrary to the opinion of some clinicians, convulsions (oil of wormwood and strychnine) *per se* did not produce cataracts.

---

[1] Luckhardt, A. B., and Blumenstock, J., *Am. J. Physiol. Proc.*, 1923, lxiii, 3, 406.

[2] Graefe-Saemisch Handbuch der gesammten Augesheilkunde, Pathologie und Therapie des Linsensystems, 1911, p. 142.

[3] Binger, C., *J. of Pharm. and Exp. Therap.*, 1917-18, x, 105.

[4] Greenwald, I., *J. Biol. Chem.*, 1913, xiv, 87.

[5] Burge, W. E., *Arch. Ophthal.*, N. Y., 1909, xxxviii.

3669

A Simplified Technique for Hepatectomy.

J. MARKOWITZ AND S. SOSKIN. (Introduced by J. J. R. MacLeod.)

From the Department of Physiology, University of Toronto.

For the past year there has been employed in this laboratory a simplified method for hepatectomy. The technique used is an extension of Mann's method for abdominal evisceration,[1] and is briefly as follows: Through a mid-line incision the inferior *vena cava* proximal to the lumbo-adrenal vein is loosely ligated with stout linen so that about ⅘ of its lumen is occluded. The portal vein, at its point of bifurcation in the portal fissure, is similarly ligated proximal to its last tributary. The intestine should be mildly congested but not cyanosed, and the ligature only rarely requires to be slightly loosened.

The dogs uniformly make an excellent recovery. The liver is removed at a second operation, which is best done six or more weeks later. The structures in the lesser omentum are tied and cut, as is also the *vena cava* below and above the liver. The intestines do not become cyanosed.

The animal makes an excellent recovery, and walks normally. We have evidence that the venous return from the posterior half of the body is not impaired. The subsequent fate of the animal is the same as that following hepatectomy by Mann's three-stage operation.[2]

This is a preliminary report.

---

[1] Mann, F. C., *Ergebnisse der Physiol.*, 1925, xxiv, 379.

[2] Mann, F. C., *Am. J. Med. Sci.*, 1921, clxi, 37.

3670

Concerning the Origin of Glycuronic Acid.

SISTER MARY ADELINE. (Introduced by C. P. Sherwin.)

From Nazareth College, Louisville, Ky.

Recently considerable work has been done in order to determine the probable source of glycuronic acid in the animal body. Some authors[1] contend that glycuronic acid is an intermediary product of glucose catabolism while others[2] believe that it is an unusual product of glucose metabolism produced only under the strain required for the detoxication of some aromatic or aliphatic substance. Other experimenters[3] think that it is derived from the catabolism of exogenous or endogenous protein material, since 58.5% of protein material is convertible into glucose.

Quick[4] found that fasting dogs, fed on benzoic acid, excrete large amounts of the glycuronic acid conjugate, and that depancreatized dogs form glycuronic acid with a decrease in urinary sugar. The excretion of benzoyl glycuronic acid is accompanied by increased endogenous catabolism. These facts indicate that glycuronic acid is made more readily from glycogenetic amino acids than from glucose itself.

Using rabbits, the effect of various amino acids on glycuronic acid formation was tried. The rabbits were fed a week on carrots and a little lettuce. Then total nitrogens (Kjeldahl) and glycuronic acids (Quick's method) were determined. The experiment was divided into three parts. During the first period, menthol was fed while giving a diet of lettuce and carrots. Then food was taken away and menthol only was given, and in the last period menthol and an amino acid were fed. Two grams of menthol as a warm water suspension was administered daily through a stomach tube. The amino acids* in the third period were fed in 1 or 2 gm. doses on alternate days in conjunction with the menthol suspension. The urine was collected without catheterization in 24 hour periods, and total nitrogens and glycuronic acids were determined. Twelve amino acids and glucose were fed in this way and their influence upon the excretion of glycuronic acid was studied. The ratio of glycuronic acid to the increase in total nitrogen was noted. Five amino acids which form glucose in the animal body were used, namely: glycocoll, alanine, arginine, cystine, and glutamic acid. Glycocoll, alanine, and arginine had a tendency to increase the glycuronic output, while leucine, isoleucine, cystine and glutamic acid seemed doubtful in their action.

Valine, phenylalanine, and tryptophane, which are non-sugar-formers, have little effect on glycuronic acid increase, as might be expected. Two of the amino acids which appeared to have great influence in increasing the glycuronic acid output are tyrosine and histidine. This is rather unusual, inasmuch as tyrosine follows that path of catabolism leading to acetoacetic acid. Histidine forms neither glucose nor autocetic acid. It probably increases purine derivatives in the urine, such as, uric acid or allantoin.

---

[*] The amino acids used in these experiments were purchased from Eastman Kodak Company.

[1] Mathews, ''Physiological Chemistry,'' 1921, iii, 759. Publishers, Wm. Word & Co.

[2] Sherwin, C. P., *Physiol. Rev.*, 1922, ii, 238. Von Fürth, ''Chemistry of Metabolism,'' trans. by Smith, 1916, 318.

[3] Quick, A. J., *J. Biol. Chem.*, 1926, lxx, 59.

[4] Quick, A. J., *J. Biol. Chem.*, 1926, lxx, 397.

3671

Differential Count of Leucocytes in Vagina of Rat During Oestrous Cycle.

CHARLES G. POST, JR. (Introduced by Carl Hartman.)

From the Department of Embryology, Carnegie Institution of Washington, Baltimore, Md.

The quantitative relations of the types of leucocytes found in the vagina of the rat during the oestrous cycle are not yet clear. Long and Evans[1] state that, during the dioestrous cycle, the leucocytes are chiefly polymorphonuclear. Loewe and Lange[2] found that, though polymorphs are predominant, there are about half as many lymphocytes. No mention is made of the monocyte.

For thirty days I examined the lavages and smears of five apparently normal rats, staining the former with a dilute solution of methylene blue, and the latter with haematoxylin and eosin. Methylene blue could have been used for the smears with much less expenditure of time and energy. The types noted in the first hundred leucocytes encountered were recorded.

A clean pipette is filled with about 1 cc. of physiological salt solution and gently inserted into the vagina. The bulb of the pipette is compressed two or three times, finally released, and the pipette withdrawn. A drop of the lavage is placed on a slide and one drop of a 0.05 % aqueous solution of methylene blue added. A cover slip is applied and the lavage immediately examined. This method was first suggested by Dr. Carl G. Hartman, who found it of great advantage in his studies on the monkey. It is an easy procedure, requiring no elaborate staining technique.

There was no difficulty differentiating monocytes from lymphocytes. A large mononuclear, twice the size of a red blood corpuscle, containing a nucleus of variable crescentic shape, whose cytoplasm is packed with large granules, I considered a monocyte. The lymphocyte generally is smaller than the monocyte; its nucleus is spherical, and the cytoplasm practically clear. The polymorphs, of course, are unique.

A study of this material yielded quite different results from those obtained by Lange.[2] During the 30 days the lavages and smears of 30 metoestrous periods were examined. From the smears the following data were obtained:

Number of smears:	4	2	7	8	3	2	4
Per cent of polymorphs:	100	99	98	97	96	95	94
Number of smears:	12	9	9				
Per cent of lymphocytes:	0	1	2				
Number of smears:	4	6	11	5	4		
Per cent of monocytes:	0	1	2	3	4		

The lavages gave practically the same results as the smears. The polymorphs numbered 95 or more. The lavage may, therefore, be considered as trustworthy a method as the smear.

Conclusion: The predominant type of leucocyte in the vaginal mucosa of a normal rat is the polymorphonuclear; lymphocytes are found in small numbers varying from 0 to 2 %; and monocytes from 0 to 4 %.

---

[1] Long, J. A., and Evans, H. McL., Memoirs of the University of California, 1922, vi, 18.

[2] Loewe, S., and Lange, F., *Ztschr. f. exp. Med.*, 1926, li, 289.

3672

On the Action of Insulin on Glucose.

J. M. JOHLIN. (Introduced by G. E. Cullen.)

From the Biochemistry Department, Vanderbilt Medical School, Nashville, Tenn.

An attempt was made to repeat the experiments of Lundsgaard and Holboll[1] in producing a new form of glucose by the action of insulin upon glucose in the presence of live tissue. The glass apparatus used was entirely of pyrex glass. For the incubation, an air thermostat was carefully controlled at 37° C. Twenty-five grams of finely cut muscle tissue of guinea pigs or rabbits were used with 25 and 50 units of Squibb's insulin, and 2 grams of Pfanstiehl glucose in 100 cc. of 0.9% NaCl. In a few cases, 0.001 grams adrenalin were added. Proper precautions to maintain sterile conditions were observed. The dialyses were made through clear collodion membranes which were one to two mm. thick. The degree of rotation of the dialysate was determined with a Schmidt and Haensch polarimeter, using light which was filtered through solutions of potassium dichromate and vanadium sulphate. The chemical analyses were made both by the Hagadorn-Jensen[2] method and the micro method of Benedict[3] as modified by Peters.[4] In no case was there a marked difference between the amounts of sugar determined by chemical and physical methods. What is more important, the degree of rotation of the dialysate was not found to have changed after these solutions had stood for 16 to 40 hours. The degree of rotation of the dialysate of samples of blood, presumably high in sugar content, was found to be too small and uncertain to deserve consideration.

This is a preliminary report.

---

[1] Lundsgaard, C., and Holboll, S. A., *J. Biol. Chem.*, 1925, lxii, 453.

[2] Hagadorn, H. C., Jensen, B. N., *Biochem. Zeit.*, 1923, cxxxv, 46.

[3] Benedict, S. R., *J. Biol. Chem.*, 1925, lxiv, 207.

[4] Peters, J. P., (private communication).

3673

Destructive Distillation of Casein in a Partial Vacuum.

MAX S. DUNN.

From the Chemical Laboratory, University of California at Los Angeles.

According to recent views, proteins are made up of heterocyclic complexes known to yield cyclic anhydrides when partially hydrolyzed, and amino acids when completely hydrolyzed. Since the character of the decomposition products seems to depend upon the nature of the degradation process, the desirability of investigating the products formed by methods other than hydrolytic is apparent.

The intention in the present work was to adapt the methods of Johnson and Daschavsky[1, 2] to casein and, as far as possible, to obtain quantitative information in regard to the products of decomposition. Casein was selected as the basic material because it may readily be obtained in quantity. It was also desired to make a comparison between this relatively complete protein and silk fibroin.

The distillation was conducted in an iron pipe 24 inches long and 3½ inches in diameter. The pipe was fitted at each end with an iron screw cap. An iron delivery tube, 20 inches long and ¾ inch in diameter, was threaded into the center of one of the caps. The screw caps were rendered gas tight by coating the threads with white lead. Graphite proved to be unsatisfactory for this purpose.

Five hundred gram portions of white, finely powdered, commercial casein were heated in the iron pipe on an ordinary combustion furnace for each of the six runs. By slightly tilting the furnace, the molten material was prevented from clogging the delivery pipe. For collecting the liquid and gaseous products, 10 round bottom, side neck, Pyrex flasks were connected in series to the delivery tube. A manometer and a good water pump were attached to the terminal flask. An oil pump was used in some of the experiments but it was found to be unsatisfactory due to its failure to maintain a constant vacuum. Flasks 3, 4, and 5, containing a total of 450 cc. of approximately 3 N sodium hydroxide, caught the acidic gaseous products. Flask 5 was a safety flask. Flasks 7, 8, and 9, containing a total of 500 cc. of approximately 3 N sulfuric acid, caught the volatile basic products. Flask 10 was a safety trap.

In each of the six runs, the pipe and flasks were evacuated to about 25 mm. This pressure was maintained for about 9 hours. The pipe was first heated gently for 1½ hours, then more vigorously, and finally as strongly as possible. The visible products of the distillation collected in the following order: water vapor, white fumes, a white precipitate in the alkali, a yellow liquid, a black liquid, and a red liquid in the acid. Gases were still issuing from the pipe when the heating was discontinued.

As may be seen from the data given in Table I the weights of products formed are comparable to those obtained by Johnson and Daschavsky. It should be noted, however, that the weights of volatile products reported by these authors were not obtained directly but by difference.

TABLE I.

Products	Weights of products recovered								Products obtained by Johnson and Daschavsky
	I[a]	II	III	IV	V	VI[b]	Total		
	gm.	gm.	gm.	gm.	gm.	gm.	gm.	%	%
Heavy liquids	137.4	132.5	143.4	148.0	144.8	121.8	827.9	27.6	
Light liquids	48.7	47.5	26.3	42.7	27.2	22.3	214.7	7.2	43.0
Gases soluble in alkali	95.2	85.8	67.5	73.4	59.6	49.7	431.2	14.4	15.9[c]
Gases soluble in acids	22.2	14.8	12.6	37.4	29.0	10.4	126.4	4.2	
Residual coke	183.7	161.6	159.3	150.0	157.3	285.0	1096.9	36.5	41.0
Total	487.2	442.2	409.1	451.5	417.9	489.2	2697.1	89.9	99.9

a—Pressure rose gradually to 95 mm. Relatively low temperature was maintained throughout.

b—Relatively low temperature and about 25 mm. pressure were maintained throughout.

c—Obtained by subtracting the sum of weights of liquids and coke from the original weight of protein taken.

Qualitative tests of the sulfuric acid solution indicated the presence of ammonia and primary aliphatic amines. Ammonia was liberated when a portion of the acid solution was neutralized with sodium hydroxide and distilled. The Rimini and carbylamine reactions were used to test for amines.

CO_2, present in the alkaline solution chiefly as sodium bi-carbonate, was determined quantitatively. Barium carbonate, precipitated by the addition of an excess of barium hydroxide to 10 cc. of the alkaline solution, was washed and transferred to a two neck flask. The CO_2, liberated by the addition of 60 cc. of 6 N sulfuric acid, was collected in 50 cc. of 0.1559 N barium hydroxide by aspirating for 1 hour at 75° C., with a stream of air freed from CO_2. A correction was made for the CO_2 present in all reagents.

The 10 cc. of alkaline solution were found to contain 0.0374 g. of CO_2, the equivalent of 16.82 g. in the total volume. This weight of CO_2 is 3.1 per cent by weight of the alkali soluble gases and 0.56 per cent of the original casein.

A similar analysis was made using an aliquot portion of the original alkaline solution instead of the barium carbonate precipitate. By this analysis 19.09 g. of volatile acids, calculated as CO_2, were found. Assuming that acetic acid is the volatile acid present in addition to CO_2, 3.09 g. of acetic acid would be present in the total volume of alkaline solution. This weight is equivalent to 0.10 per cent of the original casein.

Quantitative determinations of other products will not be possible until a later time.

This is a preliminary report.

---

[1] Johnson, T. B., and Daschavsky, P. G., *J. Am. Chem. Soc.*, 1919, xli, 1147.

[2] Johnson, T. B., and Daschavsky, P. G., *J. Biol. Chem.*, 1924, lxii, 197.

3674

Microbic Dissociation III. B. C. G. (Bacillus Calmette-Guérin).

S. A. PETROFF, ARNOLD BRANCH AND W. STEENKEN, JR.

From the Research and Clinical Laboratory, Trudeau Sanatorium, Trudeau, N. Y.

We shall describe the dissociation of a tubercle bacillus of low virulence, originally of bovine origin, which was isolated by Calmette in 1908, and passed through 230 subcultures on a special bile medium. It is claimed that at the time of isolation it produced lesions in rabbits, but after long cultivation it has lost its pathogenicity for small laboratory animals. The organism is designated as "B.C.G." (Bacillus Calmette-Guérin) and for the last five years has been extensively used for vaccinating children, with the object of attaining prophylactic immunization.

The cultures in our possession were obtained from three different sources. The first was received from Dr. Watson of Ottawa, Canada, and the second was brought from Paris by Dr. Lawrason Brown in the spring of 1926. After seeding this second culture on Sauton's fluid medium, growth and cultural differences were noticed in some of the flasks, and it was dissociated into two distinct types of colonies.

However, the study reported here on dissociation was carried out with the third culture, which came direct from the Pasteur Institute, sent to us by Professor Calmette. As soon as it was received it was suspended in salt solution and filtered as described previously,[1] and inoculated on the surface of gentian-violet medium plates. After a period of 5 to 6 weeks' incubation, two distinct types of colonies were seen. One of the colonies predominated in large numbers. From one of the plates two colonies, having different characteristics, were removed and again cultivated. This procedure was repeated until dissociation was well established. It can be seen from the photographs that these two colonies have distinct cultural characteristics. Colony R is very waxy, the folds are very smooth and raised, extending from the center to the periphery. The periphery is clean cut, rounded, slightly raised and does not extend into the medium. This colony was very difficult to emulsify and suspensions prepared from it spontaneously agglutinated at 7.2 pH.

Colony S presents an entirely different picture. There are many small, irregular wrinkles which are not smooth and do not appear waxy. The periphery is very irregular and sometimes extends into the medium. This colony is very easily emulsified and the resulting suspension does not agglutinate spontaneously at 7.2 pH.

On Sauton's medium the R colony appeared as small islands surrounded by veil-like filaments. These small islands have a smooth appearance and can be easily lifted when an attempt is made to remove them from the fluid medium. On the other hand, the S colony occurs more in solid masses covering the whole surface of the fluid medium. In attempting to remove a small fraction from the surface growth, the large mass is lifted.

Biological Characteristics of the Two Colonies. Both produce a tuberculin, render inoculated guinea pigs skin hypersensitive to tuberculin (O.T.) and produce precipitins and complement fixing antibodies in rabbits following intravenous inoculation, using homologous, heterologous and Petroff's antigens.

Results of Inoculation in Animals Skin Test Negative to Tuberculin.

I. *Guinea Pig Inoculation.* (a) *Subcutaneous:* Colony R, in doses of 2 mg. dry weight (approximately 12 mg. moist weight) produces a localized nodule which is resorbed in 40 days. Colony S, in like dosage, produces a local lesion which breaks down. There is extension to the neighboring lymph nodes and one animal died of generalized tuberculosis as early as 34 days. The lesion is transmissible in series, producing generalized tuberculosis on subcutaneous inoculation in a second group of normal animals. One animal is still living after 70 days, with local lesion and palpable lymph nodes.

(b) *Left Ventricular:* Colony R. Doses of 1.25 to 2 mg. dry weight are well supported and the animals have lived as long as 150 days. One died of unknown cause in 30 days, and was free of tuberculosis. Colony S, in similar dosage, produces generalized tuberculosis and the animals died in 17 to 26 days.

(c) *Intratesticular:* Colony R. Inoculation of 1.25 mg. dry weight produced locally typical tuberculous lesions which spread to the neighboring glands and spleen and persisted in animals killed in 51 and 75 days respectively. Colony S. The same dosage results in generalized tuberculosis and death in 75 days. (d) *Intraocular:* (Anterior Chamber or Vitreous) Colony R. A localized lesion was produced with 0.2 to 0.25 mg. limited to the inoculated eye. The animals are still living after 90 days. Colony S. 0.2 to 0.25 mg. produced death from generalized tuberculosis in 42 to 91 days. (e) *Intraspinal:* Colony R. One animal died in 20 days following 0.4 mg. The cause of death was undetermined. Colony S. A like dosage caused death in 9 to 19 days of meningitis.

II. *Rabbits.* (a) *Intraocular:* (Anterior Chamber) Colony R. 0.1 mg. produces a localized caseous lesion. The animals are still alive after 90 days. Colony S. Similar dosage produced localized caseous lesions. (b) *Left Ventricular:* Colony R. Dosages of 0.8 mg. produced no lesions in 3 months. Colony S. Two animals killed 87 days after a similar dosage showed multiple caseous tubercles in both kidneys and in one, tubercles in the lung and pericardium.
(c) *Intraspinal:* Following inoculation of Colony R and S respectively, the animals are still living after 90 days.

Attempts to Raise the Virulence of the Original Whole Undissociated Strain by Rapid Passage Through Guinea Pigs. Four series of guinea pigs skin tested negative to tuberculin were inoculated in the right testicle with 1.25 to 2.5 mg. dry weight. In 1 to 2 weeks the testicle was removed, ground up and reinjected into the right testicle of other normal guinea pigs. In only one series on the second passage have we succeeded in producing a generalized lymphatic tuberculosis which is transmissible in series on subcutaneous and intraperitoneal inoculation of other pigs. Rabbits inoculated intraperitoneally with this strain, which culturally resembles the S colony of the dissociated B.C.G. strain, develop localized caseous lesions which have so far persisted 80 days.

This is a preliminary report.

---

[1] Petroff, S. A., PROC. SOC. EXP. BIOL. AND MED., 1927, xxiv, 632-634.

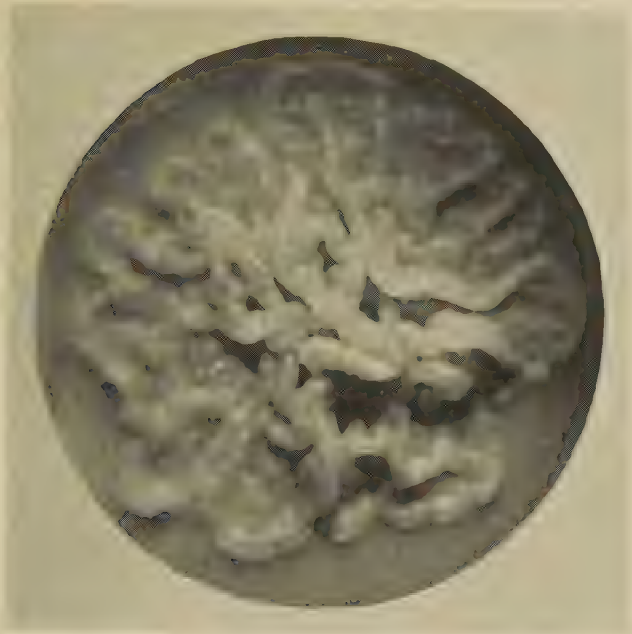


Fig. 1.
B.C.G., Colony R, on gentian-violet medium, 20 X magnification.

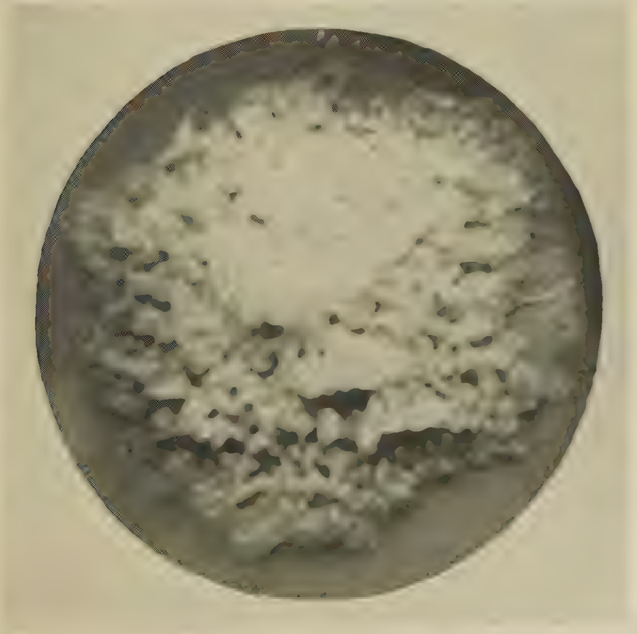


Fig. 2.
B.C.G., Colony S, on gentian-violet medium, 20 X magnification.

3675

Experimental Production of Tuberculous Intestinal Lesions in Guinea Pigs.

ARNOLD BRANCH, W. STEENKEN, JR., AND S. A. PETROFF.

From the Research and Clinical Laboratory, Trudeau Sanatorium, Trudeau, N. Y.

In several hundred autopsies of guinea pigs with generalized tuberculosis, produced chiefly by subcutaneous, intraocular, intratesticular and intraspinal inoculation of tubercle bacilli, we have failed to discover any true tubercle formation in the intestines, although hypertrophy of the lymph nodes, particularly those in the cecum, do occur. Similarly, feeding guinea pigs with suspensions of virulent tubercle bacilli for a long time, or with food contaminated with fly specks containing the organisms, was unsuccessful in producing intestinal lesions.

On the other hand, inoculation of a virulent human strain (H 37) into the left ventricle of 23 normal pigs, either in single doses of 34,000 and upwards, or in three repeated doses of 30,000 to 5,000,000 organisms, resulted in 17 animals developing definite tubercles in the lymph follicles of the gut. The cecum is most frequently involved, and next in order the ascending colon and ileum. In a few cases, feeding contaminated sputum did not alter the typical lesion. Grossly, the lymphoid follicles are enlarged, and

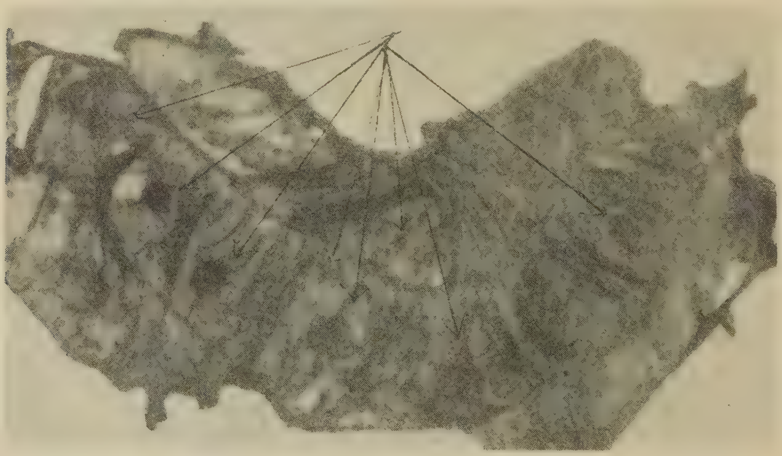


FIG. 1.

Photograph of the cecum of G. P. No. A220. This animal received a single dose of 0.25 cc. of an emulsion in saline of H 37 containing clumps and made by triturating a six weeks old colony on gentian-violet egg medium in 3 cc. of saline.

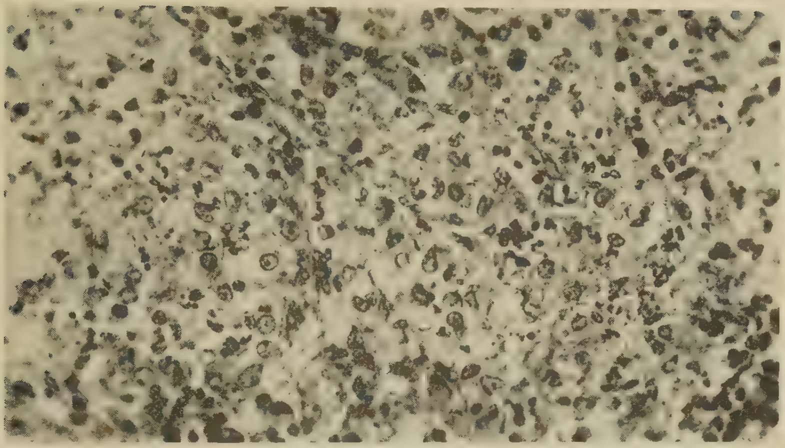


Fig. 2.

Microphotograph of one of the lesions in the lymphoid follicle of the cecum of G. P. No. E41, which died 64 days after a single inoculation of 34,000 H 37 into the left ventricle. Eosin Methylene-Blue Stain. X 400.

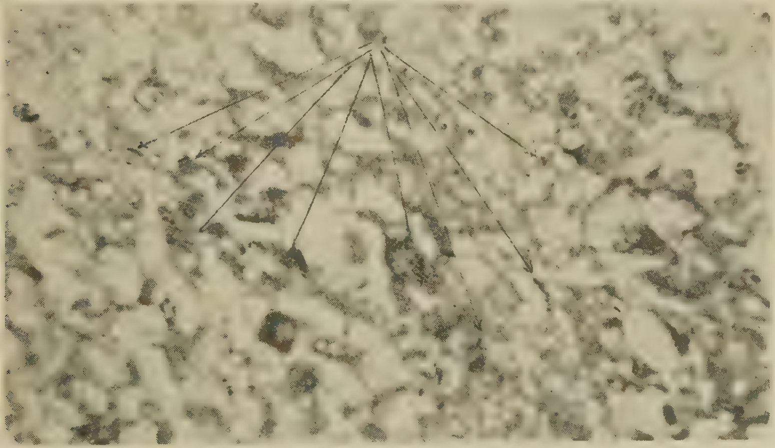


Fig. 3.

Microphotograph of the same section showing the acid-fast organisms. Ziehl-Neelsen Stain. X 900.

contain one or more small caseous tubercles. These are situated around the vessels, forming an irregular yellow tracery. Isolated tubercles are also to be found around vessels in the wall of the cecum distant from the follicles. In no case in normal animals have true ulcers been observed. The animals die in 34 to 79 days of generalized tuberculosis.

Microscopically, the lesions occur in the submucosa, especially in the lymphoid tissue of the cecum. The process is essentially a mononuclear infiltration, and cells with 2 and 3 nuclei are found. Polymorphonuclears, lymphocytes and nuclear debris make up the other constituents, while acid-fast organisms are to be found in the lesions, often in large numbers, either extra-cellular or phagocytosed. This lesion in every respect resembles the tubercles formed in normal animals in contradistinction to reinfected animals.

These observations raise the question of the hematogenous origin of infection in intestinal tuberculosis, rather than of direct transplantation through the gut wall. It is interesting that in guinea pigs, as in man, it is that portion of the gut supplied by the ileocecal branch of the superior mesenteric artery which is most often involved, and the glandular structure in this situation particularly.

This is a preliminary report.

3676

Relation of Temperature to Susceptibility of Host to Disease.

I. J. KLIGLER.

From the Department of Hygiene, Hebrew University, Jerusalem.

In a previous paper[1] experiments were reported indicating that insolation or immersion in water for short intervals lowers the resistance of guinea pigs to a trypanosome infection. Since the insolation produced a rise of 1.5° C., and, immersion a fall of 2.5° C. in body temperature, there was a possibility that the effect might be referable to temperature changes. Experiments were therefore undertaken to ascertain the effect of low and high environmental temperatures on the relative susceptibility of animals to infection.

In the present series of experiments white rats were used instead of guinea pigs. Rats of the same age and weight were injected intraperitoneally with the same dose of trypanosomes *(Tr. evansi)* and divided into two groups. One set was placed in a dark room maintained at a temperature of 28° to 30° C., and a relative humidity of 45, the other was placed in a similar room and kept at a temperature of 10° to 12° C. and relative humidity of 70 to 80. In some experiments the rats were kept at the respective temperatures throughout the duration of the test; in others, groups of rats were transferred at short intervals from the hot room to the cold room and vice versa.

Rats were considered preferable to guinea pigs for this type of experiment. In guinea pigs the disease is chronic in character, and the duration of the illness is 3 to 4 months; the criteria for differences in reaction to the virus are, therefore, the incubation period and the intensity of the infection. In rats the disease is acute and of short duration. The duration of illness can be used as a gauge of the effect of various factors on host susceptibility.

The results of the experiments thus far indicate that temperature has no effect on the course of a trypanosome infection in rats. In one experiment the animals subjected to changing temperatures succumbed much sooner than the controls at constant temperature; but these results were not duplicated in subsequent experiments. In general, no differences were observed in the cold and warm rooms.

The following experiment is illustrative: 16 rats, averaging 40 gm. in weight, were injected with 10,000 Trypanosomes each. 8 were placed in the warm room and 8 in the cold. The average duration of the disease was 17.5 days in the warm, and 18.4 in the cold room. In the former the minimum was 14, and in the latter 15 days.

Another series of experiments, also with rats, was made to ascertain the effect of temperature on an intestinal infection. Rats were fed varying doses of *B. enteritides,* and placed in warm and cold rooms respectively. In these experiments a striking difference in effect was observed. The infection was not uniformly fatal, the mortality in the cold room was 50 per cent higher than in the warm room.

The experiments are still in progress. The results, however, obtained thus far, indicate that the temperature effect as such varies with the two types of infection. It apparently exerts no effect on the course of a trypanosome infection in rats, which is essentially an acute blood infection, but has a marked influence on the progress of an intestinal infection *(B. enteritides)* in the same animal.

It is possible that in trypanosome infections of guinea pigs (which is primarily a chronic infection localized in the tissues, and only secondarily a blood infection), the temperature effect will differ from that in the rat. This possibility is now being investigated.

This is a preliminary report.

---

[1] Kligler, I. J., and Weitzman, I., *J. Exp. Med.,* 1926, xliv, 409.

3677

Observations on Hemodynamic Effect of Epinephrine in Unanesthetized Dog.

CARL A. DRAGSTEDT AND ARDATH H. WIGHTMAN.

From the Department of Physiology and Pharmacology, Northwestern University Medical School, Chicago, Ill.

The recent study by Luckhardt and Koppányi[1] on the hemodynamic action of subcutaneously injected epinephrine shows in a most striking way the influence of anesthetics upon the vascular response to this agent. They found that a pressor response was not readily obtained in animals under barbital after massage of the injected area, while good responses were obtained under paraldehyde. Ether was found to diminish or entirely suppress the pressor response, when administered by inhalation, and, when injected intravenously in small doses. Morphine apparently had no depressing effect. It appears, therefore, that anesthetics may have an effect upon the vascular response to epinephrine *per se,* or, by altering the acid-base balance of the blood.[2] As most of the previous work[3] in determining the minimal effective amount of epinephrine in altering the blood pressure has been done on anesthetized animals, or animals with brain and cord pithed, it seemed necessary to restudy this problem on the unanesthetized animal.

Under ether anesthesia animals were prepared by transplanting the carotid artery externally to the skin. Such animals are suitable for use within 5 or 6 hours, or can be used after several days. They were then placed on the table and a carotid cannula inserted. Some animals required small doses of morphine for the procedure, others not. Injections were made into the saphenous vein from a Woodyatt pump. Pure crystalline adrenalin was received from Parke Davis and Co., and solutions were made in slightly acidified distilled water, fresh for each experiment. We found a marked deterioration in the higher dilutions if made with Ringer's solution. Control injections of distilled water were made frequently. The injections were made as a rule for a period of 2 minutes, and not more than 5 to 8 cc. of fluid injected in this time.

In the normal, unanesthetized, undrugged dog we found pressor responses as the result of the minimal effective dose. This was also true for the normal animal, slightly narcotized with morphine, or, morphine and atropine. The latter dogs, as a rule, gave a somewhat smoother tracing on which smaller variations could be detected. We

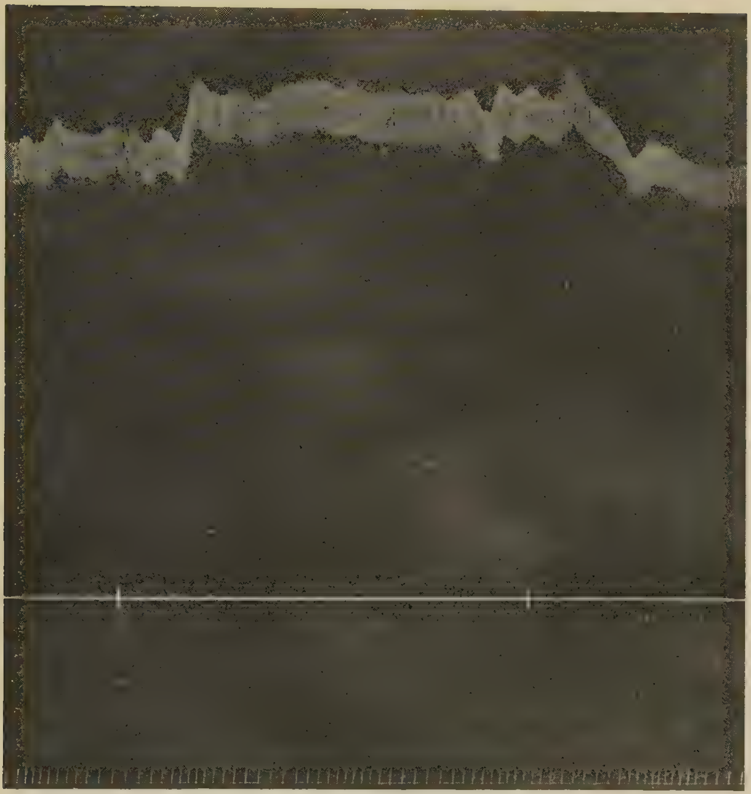


FIG. 1.

Male dog, 9 kilos, 6 hrs. after ether. Morphine Atropine narcosis. Dose: 0.18 cc. 1 to 1 million adrenalin per kilo per minute for 2 minutes.

have had pressor responses from as small amounts as 0.12 cc. of 1 to 1 million adrenalin per kilo per min. in some rather sensitive dogs, others did not give a pressor response until 0.5 cc. doses were reached. These doses are definitely under the minimal effective dose reported by Hoskins and McClure,[3] who report that the average minimal effective dose (and one which gives a depressor response) is 0.42 cc. of 1 to 1 million adrenalin per kilo per min. and that the minimal pressor amount is five times this figure. In no instance have we seen a depressor response in the unanesthetized animal. In several experiments, in which the animals were first experimented on under ether, and then allowed to recover under light morphine analgesia, we have seen depressor responses converted to pressor responses, and have then obtained pressor responses from doses which were previously ineffectual.

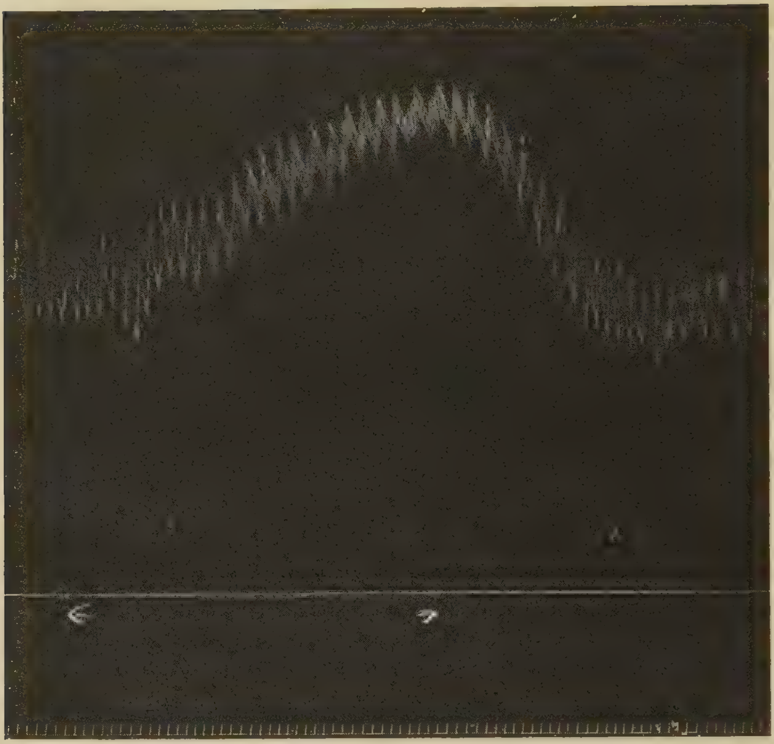


Fig. 2.

Male dog, 11 kilos. Light morphine analgesia after recovery from ether. Dose: 0.97 cc. 1 to 1 million adrenalin per kilo per minute for 2 minutes.

That these pressor responses are in reality due to epinephrine we believe to be shown by (1) the character of the response itself, (2) the absence of such responses to control injections of distilled water, and (3) a markedly augmented response seen after sensitizing the animal with cocaine.

This is a preliminary report.

---

[1] Luckhardt, Arno B., and Koppányi, T., *Am. J. Physiol.*, 1927, lxxxi, 436.

[2] Burget, G. E., and Visscher, M. B., *Am. J. Physiol.*, 1927, lxxxi, 113.

[3] Hoskins, R. G., and McClure, C. W., *Arch. Int. Med.*, 1912, x, 343.

3678

Antigens in Culture Filtrates of Bacterium Enteritidis.*

SARA E. BRANHAM.

From the Department of Hygiene and Bacteriology, the University of Chicago.

In earlier experiments[1] it was found that Berkefeld filtrates of synthetic medium cultures of *B. enteritidis* were good antigens, stimulating the production of agglutinins, precipitins, complement-fixing antibodies, and possibly antitoxins when injected into rabbits. When these filtrates were concentrated by evaporation *in vacuo,* the products obtained were better antigens than suspensions of *B. enteritidis.* Protein could not be demonstrated in this material obtained by evaporation, but when the salts were dialyzed away, and the contents of the dialyzing sac were also concentrated *in vacuo,* the non-dialyzable residue gave very faintly positive reactions for tryptophane and histidine, and a faintly positive ninhydrin reaction. Our interest was roused in the potent antigenic substances evidently present in these bacterial culture filtrates, and a study of their nature was undertaken.

Cultures of *B. enteritidis* in the synthetic medium previously described[1] were incubated for 7 to 10 days, and then filtered through Berkefeld N filters. The clear filtrates were then evaporated to dryness *in vacuo* at a temperature not exceeding 40° C. The residue, having the consistency and appearance of brown sugar, was dialyzed in a special adaptation of the apparatus devised by Hanke and Koessler[2] until it was free from demonstrable salts.

The non-dialyzable material within the parchment bag was easily separated into three crude fractions. One of these consisted of a small quantity of fine white powder which apparently contained a considerable amount of carbohydrate. This fraction has already been briefly described,[3] and further studies are being made upon it. A second fraction was a brownish water-soluble material which, although it gave none of the usual protein reactions, probably contained some protein, since it had a nitrogen content of 6 to 7 per cent. The third fraction was brownish in color, was water-insoluble, and gave a good biuret, as well as positive vanillin and Millon tests, but negative diazo and ninhydrin reactions. This fraction contained about 9 per cent of nitrogen. The nitrogen determinations were made with the micro-Kjeldahl apparatus and with the

---

* This work has been conducted under a grant from the Douglas Smith Foundation for Medical Research of the University of Chicago.

technique described by Koch.[4] Calculations of total nitrogen were made on an ash free basis.

From 100 liters of the culture filtrates, about 2 grams of the water-soluble fraction, and about 1 gram of the water-insoluble fraction have been obtained.

The toxic principle previously described as present in culture filtrates of *B. enteritidis*[5] is in the water-insoluble fraction. Solutions of this material, made in N/10 NaOH and subsequently neutralized with HCl, when injected intravenously into rabbits, produced all of the symptoms described in this earlier paper. Different lots of the material varied greatly in toxicity, just as the strength of the poison varied in different filtrates of the same strain of *B. enteritidis*. One sample was so potent that 0.1 mg. killed a rabbit weighing 2 kilograms within 2 hours; other samples required much larger doses; some produced only transient dyspnea and diarrhea with the amounts given.

These two protein-containing fractions are good antigens. Rabbits were immunized by 3 series of 3 injections given intravenously at 48 hour intervals, with a week's rest after each series. The total amounts received by the animals varied from 15 to 25 milligrams.

The sera obtained from the rabbits immunized with the water-soluble fraction agglutinated *B. enteritidis* in dilutions of 1 to 4000. They also agglutinated *B. paratyphosum B* (Schotmüller type) and *B. dysenteriae* (Shiga type) in dilutions of 1 to 400, and *B. coli* 1 to 40.

Sera obtained from the rabbits immunized with the water-insoluble fraction agglutinated *B. enteritidis* in dilutions of about 1 to 1000. They agglutinated *B. typhosum* (Rawlings) and *B. dysenteria* (Shiga) completely in dilutions of 1 to 600, *B. paratyphosum B* (Schotmüller type) and *B. suipestifer* in dilutions of 1 to 300, and *B. coli* in 1 to 100. *B. paratyphosum A, B paratyphosum B* (Aertrycke type), and *B. dysenteriae* (Flexner) were not agglutinated by any of these antisera, which were prepared with materials obtained from a single strain of *B. enteritidis*.

It would seem from these agglutination tests that these two protein-containing fractions do not possess the strict specificity of the carbohydrate-containing material. This group relation is also indicated in precipitin tests, in which varying dilutions of saline solutions of these two fractions were tested for reactivity with anti-sera prepared by immunization of rabbits with the original unconcentrated filtrates, with *B. enteritidis, B. paratyphosum A, B. paratyphosum B* (Aertrycke type), *B. paratyphosum B* (Schotmüller type), *B. suipestifer, B. typhosum,* and *B. dysenteriae* (Shiga).

Positive reactions of varying intensity were obtained with a number of these sera, although they were in no case so pronounced as with the antisera for *B. enteritidis* or its culture filtrates.

Although both of these fractions apparently possess elements in common with other members of the colon-typhoid group, they seem to be antigenically distinct from each other; *i. e.*, antisera obtained with the water-soluble fraction was precipitated by the water-soluble fractions in relatively high dilutions, but by the water-insoluble fraction was very slightly precipitated in dilutions of 1 to 10. Since these crude fractions have not been purified, it would be surprising if some cross reactions did not occur.

The relation of the antigenic activity of culture filtrates to the proteins contained in these crude fractions, and the relation of these proteins to the colon-typhoid group as a whole, cannot be determined until the materials are purified.

This is a preliminary report.

---

[1] Branham, S. E., and Humphreys, E. M., *J. Infect. Dis.*, 1927, xl, 516.

[2] Hanke, M. T., and Koessler, K. K., *J. Biol. Chem.*, 1925, lxvi, 495.

[3] Branham, S. E., PROC. SOC. EXP. BIOL. AND MED., 1927, xxiv, 349.

[4] Koch, F. C., *J. Lab. and Clin. Med.*, 1926, xi, 774.

[5] Branham, S. E., *J. Infect. Dis.*, 1925, xxxvii, 291.

3679

Comparison of Electromotive Effect of Concentration on Tissues, Proteins and Other Substances.

R. BEUTNER, P. O. HUTH AND C. K. BUSH.

From the Department of Physiology and Pharmacology, School of Medicine, University of Louisville.

Previous work in this laboratory[1] led us to conclude that proteins cannot be the essential cause of animal electricity. We then investigated the relation of salt concentration to electromotive forces which is characteristic for tissues. This seemed particularly important in relation to the recent work of Mond.[2]

Uninjured green plants show the greatest electromotive effect of the concentration, and exhibit also a striking regularity.[3] With animal organs, such as excised muscle, etc., this effect is much smaller and variable owing to unknown conditions, *but it is almost always in the same direction, viz., the dilute solution is on the positive side.* Mond,[2] working in Höber's laboratory, has tried to match this effect of concentration on tissue with gelatin; he observed + 1/1 KCl // gelatin (acid or neutral) // 1/512 KCl —0.06 volt.

It is clearly seen that the direction of e. m. f.* is *opposite* to that of almost any tissue. With alkali added to gelatin (amount not given) Mond observed, —1/1 KCL//gelatin (alkalin)//1/512 KCl +0.02 volt. This is in the same direction as tissue, but too small, since the uninjured plants (used in place of gelatin) produce an e. m. f. *four to five times* as large in this cell arrangement. It is true that most tissues exhibit a small electromotive effect of concentration, and some of them none at all. It is possible, therefore, to find some tissue which will resemble Mond's alkaline gelatin cell, however small its e. m. f. is. But it should be remembered that the number of substances which exhibit some effect of concentration is almost unlimited. This is evident from many former investigations. According to R. Beutner[3] water immiscible organic liquids will produce electromotive forces of 0.03 to 0.03 volt when used as central conductors between 1/10 and 1/1000 mol. KCl solutions. All these forces are in the same direction as Mond's alkaline gelatin cell (or tissues). Such immiscible liquids are cresol, guaiacol or other OH-compounds, acetophenon, benzophenon or other ketones; also esters like ethyl acetate, butyl acetate, aceto acetic ester, and many other similar compounds. Considering, however, that the chemical nature of all these substances is different from that of tissue constituents, we tested a true lipoid, lecithin, with no addition, for the same interval (1/10 to 1/1000 mol.). This produced 0.03 volt. These measurements, which were repeated several times with the same result, were performed by immersing a lecithin plug, first into 1/1000, and then into 1/10 mol. KCl solution, using the technique described previously.[1] The difference between these measurements then gives the figures quoted.

Such small electromotive effects of concentration are not limited, however, to water immiscible substances as middle conductors. It is quite well known that water and dilute aqueous solutions produce an effect in the same direction as tissues. The attention of physiologists has been called long ago to the fact that the insertion of a slightly acid solution between two KCl solutions of different concentration produces an electromotive force.[4] More recently Rohonyi[5] again demonstrated a small and inconstant effect of the concentration with dilute solutions of lactic acid, K_4FeCN_6, HCl, or KOH† as central conductors between 1/1000 and 1/10 mol. KCl solutions, and that these were in the same direction as Mond's alkaline gelatin cell, amounting to 0.04 to 0.06 volt (extrapolated). We have corroborated these findings by new experiments with our own technique,[1] using now as central conductor between 1/1000 and 1/10 KCl *a plug of agar,* either with or without traces of acid and alkali in it. In this way we have observed the same effect and the same magnitude or even higher than Rohonyi or Mond did with alkaline gelatin or tissue, as the following figures show. One per cent agar gel with no addition as a central conductor between 1/10 and 1/1000 molecular KCl = solutions: 0.02 volt. With a slight addition of an acid (viz. 0.1 cc. 1/1 normal HCl to 50 cc.) the e. m. f. is 0.006 volt. With a slight addition of an alkali, the e. m. f. is 0.015 volt. Mond's alkaline gelatin cell would exhibit 0.02 volt for the interval 1/10 to 1/1000. (This also shows that acid or neutral agar do not produce opposite effects like gelatin; the same finding had been reported by Fujita[7]). Similar results were obtained with other non-protein colloids such as starch paste or kaolin in the place of agar.‡

---

* e. m. f. = electromotive force.

† By comparing these experiments of Rohonyi with Mond's alkaline gelatin cell, we may conclude that the latter acts due to its alkali content alone, that the gelatin plays no part.

Summary: It is more difficult to find a material which fails to exhibit any electromotive effect of the concentration than one which does. (Concentr. KCl = solution or similar salt solutions would be about the only kind of central conductors available.) It would be useless, therefore, if we tried to identify the small effect of the concentration on the e. m. f. of animal tissue, which ranges from 0.01 to 0.06 volt, with that of any definite substance or material. Almost anything will work as a model for animal electricity if the low electromotive effect on animal tissue is chosen as criterion. There is nothing typical about Mond's alkaline gelatin cell to justify its use as a model. Moreover, it is doubtful whether its action is not due to the alkali alone.

---

‡ Experiments more recently described by Michaelis and his co-workers[7] further illustrate the fact that almost any material may serve as a bioelectric model as far as a small effect of concentration is concerned. Fujita,[6] Michaelis and Kokan[7] have measured the e. m. f. produced by such materials as parchment, caoutchouc, wax, etc., used as central conductors between 1/10 and 1/100 molecular KCl solutions. They found for parchment and paraffin about 0.01 volt, for wax and mastix about 0.02 volt, for caoutchouc 0.03 volt (As the range of concentration is ten times smaller here, the voltage should be doubled for comparison with the measurements cited.) Loeb (in his well known book on colloidal behavior) finds an e. m. f. of 0.02 volt for highly acid gelatin between 1/1000 and 1/10 KCl (extrapolated). This is also not larger than the effects on the majority of materials quoted above. Loeb's figures are not comparable with those of Mond, owing to his entirely different method, and mainly because equilibrium is established at the surface of the gelatin.

The maximal electromotive effect of the concentration as seen in plants is the only suitable object for artificial reproduction, as its magnitude is much higher, ranging from 0.09 to 0.1 volt for the interval 1/1000-1/10 mol. KCl, and particularly because in this case only a somewhat limited number of selected substances is exclusively capable of reproducing it. Such substances are: salicylic aldehyde, nitrobenzene solutions of salicylic acid, picric acid, fatty acid, lecithin, or solutions of these acids in some other solvents like guaiacol,[3] also nitrocellulose (collodion) in an air dried state (according to recent investigations of Michaelis and Fujita.[8] As an example, the effect may be quoted which is observed with a guaiacol + oleic acid mixture as central conductor between 1/1000 and 1/10 KCl; this is 0.1 volt, a value about five times as large as that given by Mond for his alkaline gelatin cell.[3])

---

[1] Beutner, R., and Menitoff, A., PROC. SOC. EXP. BIOL. AND MED., 1927, xxiv, 462.

[2] Mond, R., *Pflüger's Archiv.*, 1924, cciii, 247.

[3] Beutner, R., *Entstehung electr. Stroeme in Geweben Stuttgart*, (F. Enke) 1920. *J. Am. Chem. Soc.*, 1913, xxxv, 344, and 1914, xxxvi, 2046. *Am. J. Physiol.*, 1913, xxxi, 343.

[4] Blom, Oker, *Pflüger's Archiv.*, 1901, lxxxiv, 191.

[5] Rohonyi, *Biochem. Zeitchr.*, 1914, lxvi, 231 and 248.

[6] Fujita, A., *Biochem. Zeitschr.*, 1925, clix, 370.

[7] Fujita, A.; also Michaelis, L., and Dokam, S., *Biochem. Zeitschr.*, 1925, clxii, 255 and 258.

[8] Michaelis, L., and Fujita, A., *Biochem. Zeitschr.*, 1925, clvi, 47.

3680

The Effect of Hyper- and Hypotonic Solutions on Oxidations by the Red Blood Cell.

G. B. RAY.

From the Department of Physiology, Western Reserve University.

It has previously been reported[1] that under the proper conditions of nutrient supply, the adult red blood cell has a well defined metabolism. At the same time it was shown that dialyzed solutions of hemoglobin have the power to oxidize the same substrate, sodium lactate to CO_2. While the oxidation by the red cell is of long duration and considerable magnitude as compared with the same activity in the case of hemoglobin, one may suppose the blood pigment is associated with this oxidative activity of the cell. That this is the case is shown by the fact that loss of oxygen by the hemoglobin of the cell results in a diminished production of CO_2.

That there is a relationship between structure and oxidative activity appears to be beyond question.[2] This relationship becomes of peculiar interest in connection with the rôle of hemoglobin in oxidations by the red blood cell. If the hemoglobin of the cell is upon the cell surface, and, the assumption is correct that the blood pigments play a rôle in the oxidation by the red cell, it is conceivable that any change in the surface of the cell would produce a profound effect in the metabolism. This, of course, is dependent on the fact that the cell-solution interface is the one functioning in the process of metabolism. If this is true an increase in surface area such as would be brought about by the treatment of the cells with a hypotonic solution should cause an increase in oxidation. Conversely, the exposure of cells to a hypertonic solution should decrease the surface area with a decrease in oxidation. Suppose, on the contrary, that it is not the cell-solution interface that is active but the interfaces within the cell. In this case the hemoglobin may be conceived to be adsorbed on the surface of the colloidal matter within the cells. The result would be the direct opposite to that postulated above. The exposure of the cell to a hypotonic solution would result in the dilution of the fluid cell contents and a consequent loss of material from the surface through a redistribution of adsorbed material. A decrease in oxidation, provided these are the active surfaces, would be the result.

With these arguments in mind the following experiments were carried out upon the effect of hyper- and hypotonic solution on the metabolism of the red blood cell. Solutions of M/12.5 sodium lactate were made isotonic with the following concentrations of NaCl: 0.5, 0.7, 0.9, 1.1, and 1.3 per cent. This was done by the addition of NaCl. These solutions were placed in flasks, covered by a layer of paraffin oil and the whole sterilized in the autoclave at 15 pounds pressure for fifteen minutes. They were then stored in the cold and were warmed to 25° C. when used and maintained at this temperature throughout the course of the experiment. The blood cells were taken from dog's blood treated with sodium oxalate. They were washed three times in sterile saline, care being taken to preserve the original volume.

After a thorough oxygenation with room air the cells were added to the lactate solution in the proportion of 20 cc. of cell suspension to each 100 cc. of lactate solution. All concentrations were tested at the same time so that variations in temperature would exert the same influence on all. The oxygen content of the solution was determined at the start and at the end of the experimental period (24 hours) by the Van Slyke method. The total volume of the cells was determined at the end of the experiment either by the hematocrit or the conductivity method of Stewart.[3] The following table presents a summary of the experimental results. The data are the average of those obtained in 5 experiments. In all cases the reaction of the cells in 0.9 per cent saline has been taken as the normal amount, 100 per cent in the table.

TABLE I.

NaCl Equivalent of the Solution	Volume of Cells Per Cent	Per Cent of O_2 Consumed
0.5	115	62
0.7	107	90
0.9	100	100
1.1	87	80
1.3	78	50

In all cases the reaction of the solution was pH 6.9.

It may be seen that the variations in the tonicity of the solution decreases the amount of oxygen consumed during the period of the reaction, and, that the solution isotonic with the blood is the most favorable for the reaction. In the light of the above discussion, provided the assumptions are correct, it is difficult to conceive of the surface of the cell being an important factor in the oxidations by the cell. At the same time it is evident that disturbances in structure profoundly influence the oxidations of the cell, as might be supposed.

---

[1] Ray, G. B., *Am. J. Physiol.*, 1927, lxxxii, 405.

[2] Warburg, O., *Ergeb. Physiol.*, 1914, xiv, 253.

[3] Stewart, G. N., *Am. J. Physiol.*, 1924, lxix, 531.

3681

Effect of Starvation on Healing of Rickets in Rats.

P. G. SHIPLEY AND L. E. HOLT, JR.

From the Department of Pediatrics, Johns Hopkins University.

McCollum and his coworkers[1] demonstrated in 1922, that fasting would cause active rickets to heal within 24 or 48 hours. The immediate cause of such healing seemed clear when it was demonstrated by Kramer and Howland[2] and confirmed by Cavins[3] that starvation was promptly followed by a rise in the inorganic phosphorus of the blood serum.

Some experiments which we have performed recently seem to cast doubt upon this belief, and would seem to suggest that starvation causes healing by some other means than raising the blood phosphorus.

In the course of some experiments* on the effect of salts on calcification *in vitro,* we ran a duplicate series with preparations made from rachitic rats which had been starved for 24 hours before use. The results which are shown in the accompanying table were quite different from those obtained on the control series of rachitic rats which had not been starved.

TABLE I.

Effect of Previous Starvation of Rats on Calcification of Preparations in vitro.

Condition of Rats	NaCl added to basal solution	Number of preparations	Calcification
	mM.		
Unstarved	8.5	3	++++
”	17.1	3	++++
”	34.3	3	++±
”	51.3	3	none
Starved 24 hours	8.5	3	++++
” ” ”	17.1	3	++++
” ” ”	34.3	3	++++
” ” ”	51.3	3	++++

The basal solution used had the following composition:

Na	11.9 mM.	Cl	5.0 mM.
Ca	2.5 mM.	CO_3	10.0 mM.
P	0.97 mM.	Phenol Red	0.0006%

The solution to which 51.3 mM. NaCl had been added inhibited calcification entirely with preparations made from rats kept on the McCollum No. 3143 diet, while the preparations made from the starved rats calcified perfectly.

Obviously the amount of blood clinging to a sample of sliced cartilage is negligible, and the result must therefore be attributed to some other change brought about in the cartilage by starvation.

---

* The technique used was as described in a recent paper by one of us.[4]

[1] McCollum, Simmonds, Shipley and Park, *Bull. J. H. Hosp.*, 1922, xxxiii, 31.

[2] Kramer and Howland, *Bull. J. H. Hosp.*, 1922, xxxiii, 313.

[3] Cavins, *J. Biol. Chem.*, 1924, lix, 237.

[4] Shipley, Kramer and Howland, *Biochem. J.*, 1926, xx, 379.

3682

The "Source" of the Lytic Principle.

PHILIP HADLEY AND JOHN KLIMEK.

From the Hygienic Laboratory, University of Michigan.

Our experiments involve attempts to generate lytic principle by enforcing dissociation through the use of (1) commercial pancreatin extracts unheated, (2) similar extracts heated at 60° C., or sterilized by heat, (3) peptone solutions sterilized by heat, (4) sterile distilled water, and (5) bacterial cultures unmodified by any foreign substance. Three brands of pancreatin were employed, Squibb's, Difco and Armour's. The peptone was the Parke Davis Co. "bacteriologic peptone." The cultures used comprised a stock strain of the Shiga dysentery bacillus and a strain of *B. coli* (Jordan). In both species, fully sensitive, S type cultures relatively free from O and R forms were employed.

Pancreatin extracts were prepared in sterile broth having a pH of 7.8. First, varying amounts, heated at 60° C. or unheated, filtered (Berkefeld N) or unfiltered, were placed in contact with young Shiga and coli broth culture on sterile 20 cm. agar plates. Such plates were found to tolerate loading with even 1.0 to 1.5 cc. of the extract, spread evenly over the slightly dried surface. No sign of lytic areas was observed after incubation. The same extracts, however, heated at 60° C. for 30 minutes and filtered through a Berkefeld N candle, when added in much smaller amounts to tubes of broth seeded with young Shiga culture, resulted in the generation of active lytic principle within two to six serial filtrations. Minute amounts of the principle so generated always registered directly by the production of lytic areas when the plate method was employed. The Squibb pancreatin extracts were the most effective, yielding active lytic principle in the Shiga cultures after the first filtration. This was followed by Difco pancreatin (fourth filtrate) and by Armour's pancreatin (sixth filtrate). Only the Squibb pancreatin caused the generation of lytic principle in cultures of *B. coli* (fifth filtrate). Thus, although the ability to generate quickly an anti-Shiga lytic principle was manifested by all the samples, no evidence was gained that any of the original extracts contained lytic principle, as such, in a "preformed" state.

In consequence of these findings, the suggestion of Hoder and Suzuki,[1] that the lytic units might be present in great number in pancreatin, but intimately "bound" to the organic constituents, was taken into consideration. If such a binding were possible it might be expected that, unless the pancreatin were already "saturated" with lytic corpuscles, this substance should be able to bind more of the principle which had already been produced by the use of other samples of the same pancreatin (Squibb). Accordingly, to such dry or moistened pancreatin in tubes, was added small amounts of the homologous lytic filtrate previously obtained, and the samples allowed to stand for 24 hours at incubator, room or ice box temperatures, respectively. The samples were then dissolved or suspended in sterile broth and this was employed for tests by the direct plate method. As a result, no reduction in the amount of the lytic principle could be detected; there was no evidence of absorption by the pancreatin, nor any evidence of the destruction of the lytic principle by the pancreatin, within the time allowed for the tests.

The question then arose as to the specific ingredient of commercial pancreatin that might be responsible for the influence. The enzymes were naturally considered. Accordingly, there were prepared new extracts which were then heated in the autoclave at 110° C. for 30 minutes, or in some cases at 120° C. for 20 minutes, and finally cleared by Berkefeld filtration. Here, at least, there was no question of the presence or influence of active enzymes. Small amounts of such filtered extract were added to broth tubes which were then inoculated with a few drops of Shiga broth culture. The customary rotation of procedure, incubation, filtration, addition of fresh broth, feeding, was carried out successively. No indication of lytic action of the successive filtrates was observed until the seventh filtration had occurred. The eighth serial filtrate, when tested by the plate method, gave distinct lytic areas accompanied by broad lysis. From this eighth filtrate a strong lytic agent was subsequently built up. It thus appeared that the active enzymes contained in the Squibb pancreatin could not be responsible for the power of the Squibb extracts to generate lytic activity in the bacterial cultures.

The elimination of the active enzymes of pancreatin as a factor in the generation of lytic principle suggested the use of simpler organic preparations than pancreatin. Among these, peptone was next tried. The dissociative influence of peptone solutions on bacterial cultures has several times been noted. The solutions were prepared and sterilized at 110° C. for 30 minutes. They were then seeded with a small amount of young broth culture of the Shiga bacillus and incubated. Next followed alternate feeding and filtration in series. As a result, distinct lytic action, accompanied by the production of large and typical lytic areas, first appeared in the tests of the ninth serial filtrate. From this a strong lytic agent was eventually built up.

The success attained in the foregoing tests suggested simplifying still further the conditions of the experiment; and, in the following tests, use was made of sterile distilled water added to the culture tubes. Here, the method departed somewhat from that used with the peptone and will be reported in detail in a later publication. Suffice it to say, for the present, that a battery of broth tubes received varying amounts of sterile, distilled water and were then seeded with young culture of the Shiga bacillus; or, in another experiment, with *B. coli.* After growth occurred certain of these tubes were combined, filtered and re-distributed in 10 cc. portions, after which the procedure was repeated in series. As a result, lytic action appeared in the fifth serial filtrate for *B. coli* and in the eighth for Shiga. In another test it appeared in the ninth Shiga filtrate. From this stage a strong lytic agent was built up for each species.

Since it could scarcely be expected that a bacteriophage endowed with the power of life could be present in sterilized pancreatin extracts, sterilized peptone solution or in sterilized distilled water, it seemed possible that the experimental conditions might be even further simplified and still yield positive results. Accordingly, an attempt was made to generate the lytic agent in broth cultures of Shiga organisms without the addition of any foreign substance whatever. Three tubes of Shiga broth culture, aged, respectively, 24, 36, and 80 hours, were combined in a single tube. A small volume of fresh sterile broth was added, also a few drops of young Shiga culture. The tube was then incubated overnight. In the morning it was filtered and fresh broth, together with more culture, was added. After this, the same procedures were carried through twelve series, but without any distinct manifestation of lytic action on plate tests. The fourteenth serial filtrate, however, manifested clear lytic activity on plates, and from this filtrate a strong lytic agent was evolved by further passage.

From these experiments it appears that either fresh or sterilized pancreatin extracts, or sterilized peptone solution, in none of which can any bacteriophage, as such, be demonstrated at the beginning, can serve as the incentive for the generation, or "liberation", of abundant lytic principle in broth cultures of the Shiga bacillus or of *B. coli.* It appears, furthermore, that exactly the same result can be obtained with both cultures by the employment of sterile, distilled water only. And finally, it is also demonstrated that the same result appears, but after a longer time, in cultures where no foreign substance whatever has been brought to bear on the growing organisms.

For us, the results of these and related tests, taken in conjunction with reports of a somewhat similar nature by other investigators, carry the definite implication that the bacteriophage is generated *de novo* by the bacteria themselves, under the "liberating" influence of a specific environment. We already have evidence that this "liberating" influence is closely associated with the phenomenon of microbic dissociation. What the specific environment may involve, remains for further study.

Finally, and most important, it must be added that the strains of *B. coli* and *B. dysenteriae* Shiga employed for the preceding experiments were "normal", sensitive, type S cultures, maintained uninterruptedly for many years in the laboratory collection. They are non-lysogenic, in the common meaning of this term, and are not open to the imputation of "contamination" with the bacteriophage in the d'Herelle sense.

---

[1] Hoder, F., and Suzuki, K., *Centralbl. f. Bakt.*, Abt. I, Orig., 1926, xcviii, 433.

3683

Advanced Development of Some Echinoid Plutei.

A. R. MOORE.

From the Hopkins Marine Station of Stanford University, Pacific Grove, Calif.

Employing a modification of the method of Allen and Nelson[1] for rearing echinoderm larvae, I have been able to observe the development of three Pacific coast forms. These were the sea urchins, *Strongylocentrotus purpuratus* and *S. franciscanus*, which developed to advanced pluteus stage, and the sand urchin, *Dendraster eccentricus*, which readily underwent metamorphosis.

About 20 early plutei were put into a sterilized finger bowl containing 75 cc. of sterilized San Miquel sea water, to which a drop of the culture of *Nitschia closterium* was added. The dish was then covered with a glass plate and set in a shaded place, where the temperature during the entire summer did not vary beyond the limits 15° to 17° C. Every 5 or 6 days the larvae had to be transferred to a fresh dish of modified sea water, in order to prevent their being overwhelmed with the growth of diatoms.

After 3 to 4 weeks the plutei of *S. purpuratus* and *S. franciscanus* developed a third pair of arms, into each of which a supporting skeletal spicule grew. All the arms became very long, and then deteriorated, without the animals giving indication of any steps toward metamorphosis. These larvae always rested on the bottom.

With the larvae of *Dendraster eccentricus* development was much more rapid and vigorous. Segmentation was approximately twice as rapid as in the sea urchins. In 48 hours the 4 armed plutei were formed in 10 to 12 days, and in 10 days the third pair of arms appeared. This last seemed to be a critical step because if the third pair of arms did not appear within two weeks, the larva remained juvenile. Almost immediately after the appearance of the third pair of arms, the fourth pair began to develop from the oral plate, and were complete within the following week. Subsequently, growth in size and development of the echinus element were striking phenomena. There appeared from this time on ever increasing differences in rate of development between individuals in the same culture. Until metamorphosis, the 8-armed plutei swam freely near the top or fed on the bottom. Differentiation in the sense of shortening the arms did not normally occur. The pluteus exhibited no orientation to contact, but the newly metamorphosed animal showed the stereotropic reactions of the tube feet characteristic of the adult. The earliest metamorphosis occurred 35 days after fertilization. Dimensions of metamorphosed sand urchin were: diameter, .367 mm.; depth, .334 mm. Length of pluteus before metamorphosis, .785 mm. Shortest time required for metamorphosis, 35 days.

---

[1] Allen, E. J., and Nelson, E. W., *J. Marine Biol. Assn.*, 1910, viii, 421.

3684

Nature of Hyaline Membrane in Fertilized Egg of Sea Urchin.

A. R. MOORE.

From the Department of Animal Biology of the University of Oregon, Eugene, Oregon, and the Hopkins Marine Station of Stanford University.

The inner or hyaline membrane of the fertilized egg of the sea urchin, *Strongylocentrotus purpuratus,* which serves to hold the blastomeres together, is rendered permanent by keeping the developing eggs in a solution composed of 35 cc. sea water + 15 cc. 3/8 M $CaCl_2$. Such cultures show the following facts: (1) The outer or fertilization membrane around the inert blastula disappears normally, *i. e.,* within 24 hrs. It is therefore not broken by the hatching blastula, as is generally supposed. (2) So long as the larvae remain within the Ca-membrane they retain the form of blastulae, but segmentation goes on, and eventually the blastocoel is filled with mesenchyme cells; invagination does not occur. (3) After 3 to 5 days the larvae burst the membranes by pressure; the point of breaking has no reference to the axis of the animal. (4) After escape, the larvae invaginate and form more or less abnormal plutei; the insoluble membranes lie about on the bottom of the dish. The fertilized eggs of the sand urchin, *Dendraster eccentricus,* since they have no hyaline membrane, do not show similar reactions. In the calcium sea water solution they develop normal gastrulae and plutei only a little more slowly than the controls. In this sand urchin, therefore, the blastomeres must be held together by a mechanism different from that of the sea urchin.

If the fertilized eggs of sea urchin are put into sea water which has been brought to pH 3.5 by means of HCl, the hyaline membrane disappears, and in the course of a few minutes the perivitelline space becomes filled with hyaline droplets exuding from the cytoplasm. In volume the droplets may come to equal three-fourths the volume of the cytoplasm, but neither the diameter of the egg (.072 mm.) nor of the sphere enclosed by the outer membrane (.098 mm.) is immediately altered. Therefore the droplets must largely consist of the sea water of the perivitelline space transferred into the droplets, presumably by osmosis. The whole process is suggestive of the Spiro-Henderson effect. If now the eggs are returned to normal sea water of alkaline reaction, the hyaline droplets return into the cytoplasm, *i. e.,* the process is reversible. If, however, the eggs in the acidulated sea water are centrifuged, the hyaline droplets are broken away from the cytoplasm. Such eggs upon being returned to normal sea water continue their development, but the hyaline droplets remain lying free in the perivitelline space, and when the fertilization membrane breaks, they fall out and may be seen spread over the bottom of the dish. This proves that resorption of the droplets in the uncentrifuged eggs depends upon the physical connection of the droplets with the cytoplasm. The larvae resulting from both centrifuged and uncentrifuged eggs develop to normal early plutei. Those resulting from cytoplasm minus droplets are slightly smaller in size than normal. The results prove that the hyaline substance lost from the cytoplasm in the centrifuged eggs is not essential to normal development.

The hyaline membrane behaves like an ion protein compound formed from an exudate of the blastomeres. This substance in sea water pH 8.2 reacts with Ca to form an insoluble Ca-proteinate membrane. In acidulated sea water pH 3.5, the Ca-proteinate cannot exist; consequently, the membrane disappears. The hyaline membrane, therefore, behaves like an ion-protein compound, in the sense used by Loeb.

3685

Self Differentiation of Intestine in Larvae of Sand Urchin, Dendraster Eccentricus.

A. R. MOORE.

From the Hopkins Marine Station of Stanford University, Pacific Grove, Calif.

Exogastrulae formed spontaneously, or induced by means of Herbst's lithium chloride method, develop into otherwise normal plutei. In such plutei the everted gut is differentiated clearly into intestine, stomach and oesophagus. On the free end of the oesophagus a tuft of amoeboid endoderm forms, which has adhesive properties. If the amoeboid endodermal tufts of two individuals meet they coalesce, and the walls of the two oesophageal tubes fuse so as to form a continuous tube between the two animals. This result suggests that normally the attachment of the oesophagus to the stomodeum is accomplished by a similar mechanism. In the exogastrulae the gut grows out from the anal surface of the animal at an angle, the mirror image of which (as is the case in the normal animal) would bring the end of the structure in close proximity to the stomodeum. The fact that differentiation takes place in the case of the everted gut outside the animal, removed from the influence of other tissues, except at the point of attachment, suggests that differentiation is not a result of interaction of tissues, but that it is predetermined in the primitive cells. Whether this view is correct was tested by means of microdissections.

Larvae in the gastrula stage were cut in two at various levels, across the longitudinal axis. The parts were separated, and each set aside in a watch glass to develop. Some of the diagonal cuts resulted in one-armed plutei, showing that the pattern already had been laid down in the cells of the gastrula. In some cases just enough material was contained in the vegetal piece for the formation of alimentary tissue. Such pieces showed differentiation into stomach, oesophagus and amoeboid endoderm. The oesophagus in such cases was round and not cylindrical, as in the normal. This result suggests that attachment to the more rigid skeletal structure is necessary for the proper tensions in the growing alimentary tract. The experiments prove that in the gastrula stage, approximately the vegetal third of the larva contains the elements for self differentiation of the gut.

3686

New Dietary Deficiency with Highly Purified Diets.* II. Supplementary Requirement of Diet of Pure Casein, Sucrose, and Salt.

HERBERT M. EVANS AND GEORGE O. BURR.

From the Anatomical Laboratory, University of California, Berkeley.

In a recent preliminary report[1] it was shown that female rats could attain normal adult weight and exhibit normal ovulation on the highly purified diet 519† only when a small amount of lettuce or liver was added to the daily supplement of yeast and cod liver oil. There remained some doubt as to whether Vitamine B as found in yeast (antineuritic B, plus growth B) was capable of making up the growth deficiency if the daily dose were somewhat increased. There was also naturally some question as to whether there might not be possible mal-effects in attempting to satisfy the entire energy requirements of mammals throughout the life cycle with sugar.

Vitamine B Requirements. In this paper we shall employ the term Vitamine B to signify both antineuritic B and purely growth promoting B, both being found in yeast and wheat germ. Eight groups of 3 or 4 animals each have been reared on diet 519† with daily doses of dried yeast or wheat germ as supplements for their Vitamine B requirements. It is especially important to note that we have reared all animals without access to faeces or bedding, *i. e.*, on wire bottoms. The brief summary of results given in Table 1 brings out several interesting facts. 1. The growth was strikingly uniform in all groups and was subnormal, 180 to 190 gm. being the approximate

---

* Aided by grants from the Committee for Research on Problems of Sex of the National Research Council and from the Bureau of Dairy Industry of the United States Department of Agriculture. These funds have been generously augmented by the Board of Research and by the College of Agriculture of this institution.

†Ingredients	Diet 519	Diet 520	Diet 521	Diet 522
Casein, highly purified	25.0	—	—	30.0
Casein, commercial	—	27.5	27.5	—
Sucrose, recrystallized	75.0	—	75.0	46.0
Sucrose, commercial	—	75.0	—	—
Lard, commercial	—	—	—	20.0
Salts, 185	4.0	4.0	4.0	4.5

Note: The slight increase in the amount of commercial casein is to offset its moisture and impurities. In all four diets, the nutritive ratio is approximately constant and approximately 1:4.

normal weight at 90 days of age in females from our colony. 2. Animals receiving 1.0 gm. of yeast daily were not superior in growth or ovulation to those receiving only 0.7 gm. yeast. This would appear to establish that stunted growth of animals on these diets is not due to a shortage of Vitamine B, both components of which are normally abundant in our yeast. 3. The age of maturity and early ovulation history varied greatly, and unaccountably in the different groups. In some groups (4, 8) after a fair beginning they became markedly subnormal; in others (2, 3) they were strikingly regular in spite of subnormal growth.

The Necessary Supplement for Diet 519. Our early work indicated that diet 519 was deficient in some substance other than Vitamine A, B, D, or E and that this deficiency was overcome by the addition of 0.5 gm. fresh beef liver or 10 gm. fresh lettuce daily.

Repetition of the work has fully confirmed the above findings. The number of animals has been left small because of the expense of the diet, but the results are so uniform that there is no doubt as to the validity of our early conclusions. Table 2 summarizes the results of tests on the following supplements: liver, lettuce, lard and the impurities in commercial casein.

Groups 4 and 5, with commercial casein, show little or no improvements over the 3 control groups (groups 1, 2, and 3), and it is evident that the various milk ingredients other than protein, which are in considerable amount in commercial casein, do not supplement diet 519. Groups 6, 7, 8, and 9 show the value of liver, lettuce and lard as supplements to the pure diet. In these groups, the animals all grew uniformly to the normal 90 day weight of 190 grams and showed excellent ovulation histories, whereas in some groups their litter mate sister controls exhibited very defective ovulation. The remarkable effect of lard has become the subject of special further study and is being reported at this time under separate title.[2] It seemed desirable to attempt a preliminary fractionation of lettuce leaves. The leaves were slowly dried in a warm room so that the cured product retained all of the natural green color and a fine odor. The powdered leaves were extracted with pure ether in a Soxhlet. The ether extract was evaporated to a small volume so that 1 cc. represented 1.0 g. of the dried leaf and this was evaporated on the yeast dose daily. In other cases, unextracted dry leaf powder was fed with the yeast dose. The subnormal growth of the animals receiving these supplements (Table 3) indicates a considerable destruction of the active substance present in fresh lettuce leaf had resulted from the drying process.

Reproduction and Lactation on the Pure Diets. Animals reared on diets 519 or 520 are invariably sterile by the 90th day of life, as was shown by breeding them at the occurrence of the preoestrous stage with fertile males and observing resorption gestations. But that sterility is due to low E is shown by the production of good litters when wheat germ oil is administered in sufficient dosage.

In most of our tests on reproduction and lactation on the "pure" diets, the effort to study these phenomena was always made somewhat beyond the close of the third month of life and consequently at a time when, as has just been explained, the ovulation history was poor. In these animals, consequently, oestrous had to be induced. We accomplished this by subcutaneous injection of 15 mg. of a placental extract.

After the birth of the young, the yeast dose was trebled because we had previously shown that at least thrice the maternal requirement for B is essential for the proper lactation of 6 young. To prevent the young from falling through the wire bottom, solid sheets of tin flooring were employed. No bedding of any sort was allowed, but the cages were not allowed to get cold, being held at 24° C. by thermostatic control. Although great care was exercised by us, all but 3 of the 71 young given for lactation in these 13 gestations, perished during the lactation period. (Table 4.) Evidently, then, the deficiency of the "pure" diets is shown in an especially magnified way by attempted lactations on those diets.

Reproduction and Lactation on the Pure Diet 519 Supplemented by Lettuce. The females in Table 2, group 7, had received lettuce all their lives and were hence not sterile. They were mated when about 4 months old and good litters were produced. A brief record of their lactation performance is given in Table 5. Here also, during lactation, Vitamine B was increased by feeding 3 times the usual dose of yeast. The lactation performance may be said to be far from perfect, but the mortality was not high and the young are still in fine health, though small. These second generation young, therefore, repeat nicely our experience with their parents. They are small, glossy coated, active individuals with every appearance of health, save again for the two striking features of sexual subnormality and incomplete growth.

Summary. 1. The "pure" diet of casein (Van Slyke), sucrose (recrystallized) and salts 185, is not adequate for normal growth although supplemented by very high levels of vitamines A, B, D, and E. In some cases, with the defective growth, ovulation is strikingly regular though in other cases it becomes defective after a month or two. 2. Lactation fails with the "pure" diet. 3. The growth and ovulation deficiencies are corrected by small daily doses of lettuce or liver, or by the inclusion of lard in the diet. 4. With the additional supplement of lettuce, females produce good litters and they successfully wean them if the yeast dose is adequate. 5. The second generation animals thus successfully weaned, repeat the performance of their parents. Though in perfect health on the "pure" diets, they are always greatly retarded in growth. 6. These experiments, twice repeated, appear to leave no room for doubt that the particular "pure" diet in question either demands an extraordinary and unique amount of some one of the known vitamines or else another and unknown member (F or H) of the vitamine class. Although care to detect them was exercised, the specific criteria for belief in inadequacy in A, D, and E were not encountered. The absence of any change with increased B dosage appears to speak decisively against the complicity of B and to hence leave the second hypothesis as the only alternative.

TABLE I.

Showing the lack of any appreciable effect of various sources and levels of Vitamine B on the constant subnormal growth produced by Diet 519.

Group	Diet	Age at opening	Age at first estrus	Ovulation cycles	Wt. at 90 days
		days	days	days	gm.
2	519				
	0.7 g. yeast (Fleishman)	43	43	30, 6, 5, 5, 5, 5, 6, 5, 6, 5, 7	131
	3 drops cod liver oil	39	39	20, 15, 6, 6, 7, 10, 18, 6	124
	(Patch)	69	69	6, 5, 5, 5, 4, 6, 4, 4, 6	138
		38	38	12, 3, 7, 9, 5, 5, 5, 5, 5, 5, 5, 5, 5, 5, 5	144
3	519				
	0.7 g. yeast (N'western)	46	51	5, 5, 5, 5, 5, 4, 6, 5, 5, 5, 4, 5, 6, 5, 5	152
	3 drops cod liver oil	41	47	10, 6, 6, 4, 4, 7, 5, 5, 5, 5, 4, 6, 5, 4	152
	(Patch)	47	47	6, 5, 6, 8, 5, 11, 6, 5, 5, 6, 5, 5	157
4	519				
	1.0 g. yeast (Fleishman)	44	48	5, 8, 7, 6, 13, 8, 5, 7, 8, 13, 34	132
	3 drops cod liver oil	45	45	5, 5, 5, 4, 6, 5, 5, 6, 5, 7, 6, 6, 10, 10, 25	136
	(Patch)	42	42	10, 6, 23, 8, 7, 5, 61	142
6	519				
	0.6 g. fresh wheat germ	60	69	6, 10, 11, 7, 9, 17, 23, 10	107
	3 drops cod liver oil	51	51	5, 5, 5, 5, 5, 5, 6, 6, 5, 6, 6, 7, 7, 34	120
	(Patch)	46	57	5, 5, 7, 6, 6, 6, 6, 8, 6, 24, 10	150

TABLE II.

Showing the value of various materials as a supplement to diet 519. (The first three groups constitute controls on the unmodified diet 519 and consist of litter mate sisters of animals distributed in other groups.)

Group	Diet	Age at opening	Age at first estrus	Ovulation cycles	Wt. at 90 days
	519	days	days	days	gm.
1	0.8 g. yeast	43	72	No cycles	130
	3 drops cod liver oil	47	48	19, 22, 35	143
		53	57	4, 5, 7, 90	150
4	521 (identical with 519 except for the employment of commercial casein)				
	0.8 g. yeast	43	50	6, 5, 5, 6, 13, 6, 4, 6, 5, 5, 16, 35+	148
	3 drops cod liver oil	52	56	6, 6, 5, 5, 5, 5, 5, 5, 5, 5, 5, 14, 34+	164
		61	61	16, 16, 13, 56+	149
5	520 (identical with 519 except for the employment of commercial casein and sucrose)				
	0.7 g. yeast	78	80	9, 18, 20	162
	3 drops cod liver oil	71	72	7, 4, 6, 5, 11, 23	132
		78	80	6, 40	138
		68	72	7, 5, 17, 6, 8, 12	138
		76	—	— no ovulations	138
		63	63	5, 6, 6, 4, 5, 5, 7, 6, 6	156
	519				
6	0.8 g. yeast	54	55	7, 8, 9, 22	190
	3 drops cod liver oil	41	42	6, 4, 5, 4, 6, 5, 5, 5, 5, 4, 4, 5, 5, 4	189
	½ g. liver	Wheezy 56th d. Killed. Wt. 122g. Growing rapidly			
	519				
7	0.8 g. yeast	43	43	7, 6, 6, 5, 6, 4, 6, 5, 5, 5, 5, 5, 6, 5	190
	3 drops cod liver oil	43	43	11, 5, 7, 5, 4, 6, 6, 5, 8, 11, 5	194
	10 g. fresh lettuce	49	49	12, 5, 5, 5, 5, 5, 5, 5, 5, 5, 5, 5	179
9	522 (identical with 519 save for the decrease in sugar and the addition of commercial lard)				
	1.0 g. yeast	41	42	5, 5, 6, 5, 6, 5, 5, 6, 6, 6, 8, 5, 5	189
	3 drops cod liver oil	41	48–	6, 7, 5, 5, 5, 7, 5, 7	182
		43	43	4, 8, 5, 7, 6, 5, 5, 5, 5, 6, 18	174

TABLE III.

Showing partial efficacy of dried lettuce and lettuce leaf oil as a supplement to diet 519.

Group	Diet	Age at opening	Age at first estrus	Ovulation Cycles	Wt. at 90 days
	519	days	days	days	gm.
1	0.7 g. yeast	46	46	8, 7, 7, 6, 6, 6, 5	151
	3 drops cod liver oil	58	62	10, 7, 7, 7	150
	dried lettuce	34	34	5, 13, 5, 4, 6, 10, 5, 5, 5, 4	173
	519				
2	0.7 g. yeast	67	71	4, 7, 5, 7, 5	158
	3 drops cod liver oil	36	36	11, 26, 5, 6, 5	129
	ether extract of 1 gr.	39	39	33, 8, 6, 9	147
	dry lettuce	41	70	5, 5, 7, 6	137

TABLE IV.

Showing the completion of gestation but failure in lactation of mothers on pure diets (519, 520, 521).

Wheat germ oil (10 drops daily) was given during gestation and the yeast dose trebled during lactation.

No. of young born		Average birth wt. of living	No. of young given for lactation	No. of young weaned	Notes
Alive	Dead				
		gm.			
9	0	3.5	6	0	Destroyed by mother on 3rd day
3	0	5.0	3	0	Found dead by 13th day
8	0	4.5	6	0	Found dead by 13th day
7	0	6.0	6	0	All destroyed by 6th day
8	0	4.5	6	0	All dead by 6th day
7	0	5.0	6	0	All destroyed by 2nd day
8	0	5.4	6	0	All destroyed by 2nd day
8	0	5.0	6	0	All gone by 5th day
5	0	5.2	5	2	3 gone by 16th day
5	0	5.0	5	1	4 gone by 16th day
7	0	4.7	6	0	All gone by 7th day
8	0	5.0	6	0	All gone by 10th day
4	0	5.5	4	0	All gone by 6th day

TABLE V.

Showing the Beneficial Effect on Lactation of Adding a Small Amount of Fresh Lettuce Leaf (10 grams) daily to the Pure Diet 519.

(Casein pure 25, sucrose pure 75, salts 4; separately, 3 drops of cod liver oil on 2.1 grams whole dried yeast.)

No. of young born living	Average birth weight	No. of young given for lactation	No. of young weaned	Average wt. on 21st day of life
	gm.			gm.
7	5.0	6	3	30.0
4	4.8	4	4	24.0
5	6.0	5	5	28.0

TABLE VI.

Showing the growth of second generation males and females on diet 519 plus 0.7 g. yeast daily and 3 drops cod liver oil. (Always on wire bottom.)

Age at opening	Age at 1st estrus	Ovulation Cycles	Wt. at 45th day	Wt. at 60th day
days	days	days	gm.	gm.
55	—	None at 65 days	83	108
59	—	” ” ” ”	78	114
48	—	” ” ” ”	67	102
48	—	” ” ” ”	62	106
51	—	” ” ” ”	66	108
54	—	” ” ” ”	60	102
48	48	8	62	108
53	53	No more at 65 days	63	88
—	—	———	79	126
—	—	———	64	100
—	—	———	59	86
—	—	———	72	132

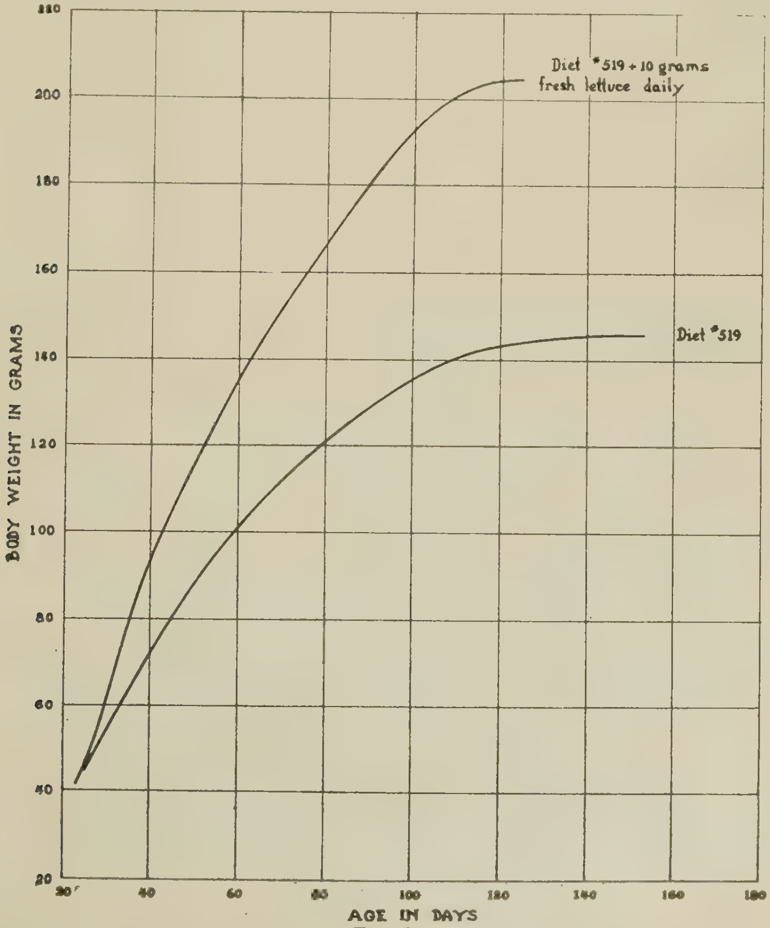


FIG. 1.

Composite growth curves of females showing the invariable stunting which occurs with the highly purified Diet 519 and generous supplies of the known vitamines contrasted with the normal growth produced in litter mate sisters held on the same diet supplemented only with 10 gm. daily of fresh lettuce leaf substance.

Diet 519—Casein, purified, 25; sucrose, recrystallized, 75; salts 4; yeast or wheat germ daily, 0.7 grams; cod liver oil daily, 3 drops.

---

[1] Evans, H. M., and Burr, G. O., PROC. SOC. EXP. BIOL. AND MED., 1927, xxiv, 740.

[2] Evans and Burr, The Beneficial Effect of Fat in the Diet and the Evidence for a New Dietary Factor for Growth and Ovulation.

3687

A Powerful Bacteriophage Against Hemolytic Streptococci of Erysipelas Origin.

GREGORY SHWARTZMAN.

From the Laboratories of the Mount Sinai Hospital, New York.

Attempts by several authors to obtain an active lytic principle against streptococci were without definite results. Successful attempts with single strains were reported by Piorkowsky,[1] Dutton,[2] Hadley and Dabney,[3] Clark and Clark.[4] Only two strains were of human origin (Piorkowsky, Dutton). It remains yet to decide as to whether streptococci as a genus are susceptible to the bacteriophage phenomenon, or whether the few strains of streptococci, in which this phenomenon was observed, carried a factor, not common to other streptococci as a genus, which enabled them to be affected by the phage. In this work attempts were made to obtain a lytic principle against human pathogenic streptococci by means of various lytic principles, namely: anti-*B. coli* phage; anti-*staphylococcus* phage; lytic principle isolated by Clark and Clark from activated sludge against a strain of rabbit *streptococcus hemolyticus*. Numerous human strains proved to be entirely resistant towards these phages. One hundred and two strains were tested against Clark and Clark's phage. These human strains also completely lacked the power of regenerating these principles. Since the power of regeneration is essential for obtaining classical bacteriophage phenomenon, various resistant strains were placed under supposedly favorable conditions to enable them to perform this function. All these attempts failed. An effort was then made to "train" the pathogenic strains to regenerate bacteriophage by "adapting" the microorganisms to the lytic principles, instead of the usual method, of adaptation of lytic principle to the microorganisms. Various strains of human pathogenic streptococci chosen for this experiment were subcultured every 24 hours in broth containing 1:10 dilution of lytic principle. After several such passages the cultures were inoculated into flasks of broth, incubated for 24 hours, and 0.5 cc. of each of these filtrates tested against homologous normal cultures of streptococci. Strains of *erysipelas streptococci* "trained" in Clark and Clark's phage acquired the property of regenerating a powerful lytic principle against normal cultures of these streptococci. The lytic principle thus obtained corresponded fully to the classical bacteriophage in every detail. This principle also showed a remarkable specificity. Sixteen strains of *streptococcus erysipelatis,* out of 21 tested, were lysed in dilution 1×10^{-8} cc. by this phage. Sixty-four strains from other sources remained entirely unaffected by *streptococcus erysipelas* phage. The method failed to procure a phage against other pathogenic streptococci.

---

[1] Piorkowski, G., *Med. Klin.*, 1922, xviii, 474.

[2] Dutton, L. O., *J. Inf. Dis.*, 1926, xxxix, 48.

[3] Hadley, P., and Dabney, E., Proc. Soc. Exp. Biol. and Med., 1926, xxiv, 13.

[4] Clark, P. I., and Clark, A. S., Proc. Soc. Exp. Biol. and Med., 1927, xxiv, 635.

3688

Gastro-Intestinal Motor Response to Vagus Stimulation after Nicotine.

MICHAEL G. MULINOS. (Introduced by C. C. Lieb.)

From the Department of Pharmacology, College of Physicians and Surgeons, Columbia University.

While studying the effect of massive doses of nicotine on the enteric nervous mechanism of dogs, Thomas and Kuntz[1] stimulated the vagi after giving large doses of nicotine. The response of the intestine was not abolished by these large doses of nicotine, but, on the contrary, were greater and more constant than before nicotine was administered. This was apparently contrary to the generally accepted conclusion of Bayliss and Starling.[2]

The present report is based on 28 experiments on cats, 19 females and 9 males, weighing from 2.0 to 4.1 kilograms. They were narcotized with ether, and the brain and spinal cord destroyed by pithing. Artificial respiration was administered through a tracheotomy tube. Both vagi were exposed, but only the right was electrically stimulated after a primary testing of the effect of both, Fig. 1. A rubber balloon was inserted into the intestine disto proximally, and a record of the activity obtained by means of a water manometer. The nicotine used was made up as a 5 per cent solution by weight of the alkaloid in 0.9 per cent saline and neutralized to pH 7.4 with HCl. Nicotine salicylate was used in 3 experiments. The solution was injected into the femoral vein.

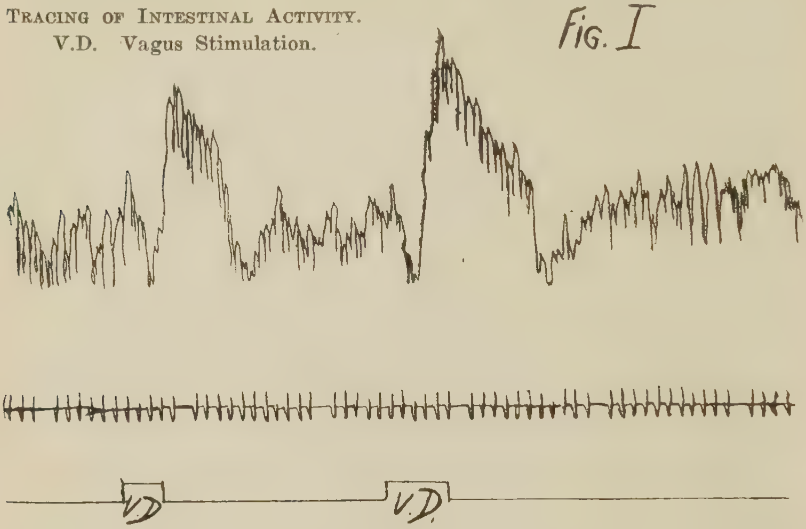


FIG. 1.

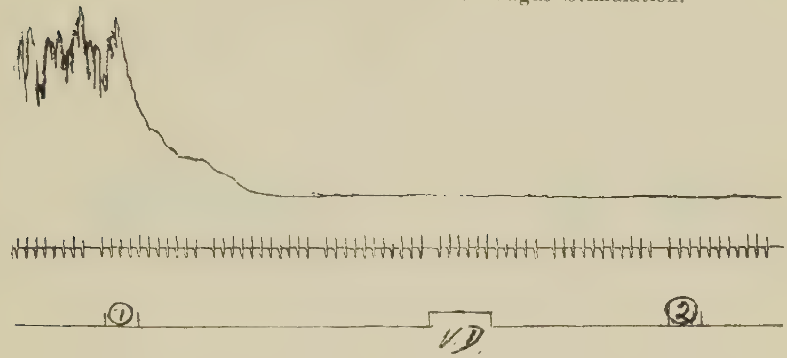
Cat. Balloon water manometer record of intestinal activity. Tracing shows effect of vagus stimulation before administration of nicotine.

Three series of experiments were done as follows: (a) 11 experiments with same procedure as described above. (b) 9 experiments with both adrenals removed. (c) 8 experiments with both adrenals and semi-lunar ganglia extirpated.

As the results were essentially the same, whether the adrenals and semi-lunar ganglia were present or removed, they will be presented together.

Nicotine given intravenously causes a cessation of motility and a drop in tone of the cat's intestine. If the vagus response was good before the injection of the nicotine, it invariably disappeared with the first injection, which was always between 1 to 10 mg. per kilo of body weight, Fig. 2. Subsequent injections had no further effect upon motility, tone or reaction to vagus stimulation of the intestine, until a total amount of 20 to 60 mg. of nicotine per kilo had been injected, (Fig. 3).

Fig. II



TRACING OF INTESTINAL ACTIVITY.
(1) and (2) 1st and 2nd injections of nicotine to 10 mg./Kg.
V.D. Vagus Stimulation.

FIG. 2.

Same animal as in Fig. 1. Tracing shows cessation of intestinal activity with loss of vagus response and inocuousness of nicotine injection at (2), following the initial intravenous injection of nicotine at (1).

Fig. III

TRACING OF INTESTINAL ACTIVITY.
(4) 4th injection of nicotine; total now 40 mg./Kg.
V.D. Vagus Stimulation.

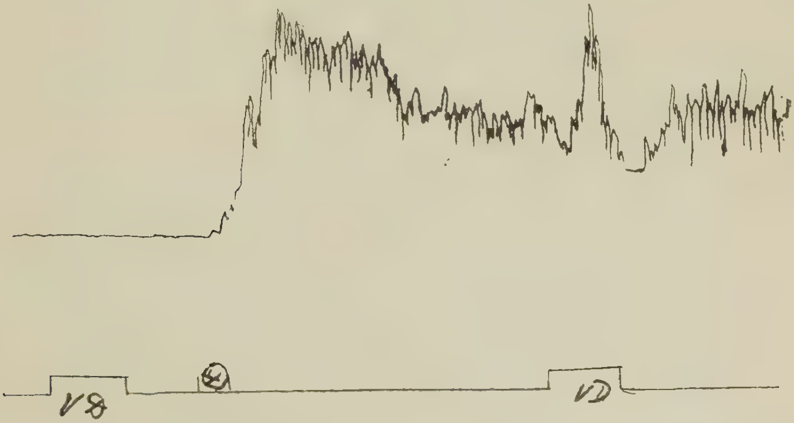
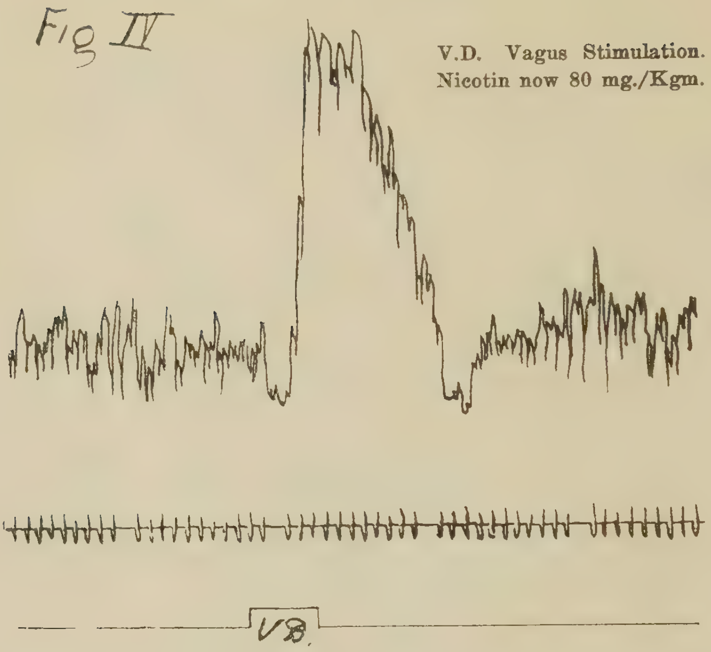


FIG. 3.

Same animal as in Fig. 2. Tracing shows continued loss of vagus response thirty-five minutes after the first injection (Fig. 2 (1)) as above. It shows stimulation of the intestine at (4), forty-two minutes after the first injection; as well as a return of vagus response a few minutes later.



V.D. Vagus Stimulation.
Nicotin now 80 mg./Kgm.

FIG. 4.

The same animal as in Fig. 3. Tracing shows exaggerated response to vagus stimulation after 80 mg. of nicotine per kilo had been given.

When this latter concentration was reached, the injection of nicotine was followed by an increase in intestinal tone and activity. These increased in strength and duration as the total amount injected became greater, though the individual doses and the time interval between them remained approximately the same, Fig. 4.

When the concentration, above described, was reached, there was also a return to vagus response, which became greater with each injection. Finally a point was reached (50 to 100 mg. of nicotine per kilo) when the vagus response was greater and of longer duration than the original one previous to the first nicotine injection, Fig. 4. In every case where the primary response to vagus stimulation was good we obtained a return of the activity after the higher concentration of nicotine had been injected. There was no primary response to vagus stimulation, throughout the experiments, though the response to nicotine described in the preceding paragraph was always present.

For the theoretical discussion of the results, the reader is referred to the paper by Thomas and Kuntz.[1]

The conclusions seem justified that:

(1) All effects of the vagus on the intestine of the cat are com-

pletely and permanently abolished by doses of nicotine from 1 to 15 mg. per kilo of body weight.

(2) These doses depress the intestine, as shown by the drop in tone, and the cessation of activity of that organ.

(3) Doses larger than 15 to 60 mg. per kilo of body weight brought about a return of the previous activity and a response to vagus stimulation, whether the drugs were given in one or divided doses.

(4) Neither the splanchnic ganglia nor the adrenals are responsible for the depression of the intestine or for the loss to vagus stimulation following the small doses of nicotine, because their removal causes no change in the reaction of the nicotine.

---

[1] Thomas, J. E., and Kuntz, A., *Am. J. Physiol.*, 1926, lxxvi, 598.

[2] Bayliss, W. M., and Starling, E. H., *J. Physiol*, 1899, xxiv, 99.

3689

Formation of Lactic Acid in Excised Organs.

HAROLD E. HIMWICH AND SHELDON A. JACOBSON.

From the Department of Physiology, Yale University Medical School.

These experiments were undertaken to determine whether excised tissues would form lactic acid under anaerobic conditions. Paired organs of dogs were studied. One of each pair was rapidly triturated in chilled alcohol to prevent post-mortem glycolysis. The other was triturated in Ringer's or buffered phosphate solution (pH

TABLE I.

Lactic Acid in Paired Organs Before and After Incubation.

Dog No.	Organ	Lactic Acid Per Cent	
		Before	After
59	Testis	0.017	0.25
27	”	0.037	0.22
141	”	0.006	0.18
59	Kidney	0.054	0.32
27	”	0.068	0.20
141	”	0.029	0.15
59	Parotid	0.051	0.14
27	Thyroid	0.075	0.16
141	”	0.028	0.090
27	Cerebrum	0.118	0.196
59	Submaxillary	0.086	0.178

8) and then incubated at 37.5° C. for 3 to 10 hours to allow for any possible formation of lactic acid. The lactic acid, after extraction and purification by Meyerhof's procedure,[1] was determined by the oxidation method, as modified by Friedemann, Cotonio and Shaffer.[2]

In every organ studied (kidney, testis, parotid, submaxillary, thyroid, cerebrum) a definite increase of lactic acid was obtained after incubation, an amount ranging from 1 2/3 to 30 times that originally present.

Investigation is now under way to determine the origin of lactic acid so produced.

---

[1] Meyerhof, O., *Arch. f. d. Ges. Phys.*, 1921, clxxxviii, 114.

[2] Friedemann, T. E., Cotonio, M., and Shaffer, P. A., *J. Biol. Chem.*, 1927, lxxiii 335.

3690

Glycolysis in Semen.

J. F. McCARTHY, C. T. STEPITA, M. B. JOHNSTON AND J. A. KILLIAN.

From the Department of Urology and the Department of Laboratories, New York Post-Graduate Medical School and Hospital.

In fresh specimens of semen obtained from 50 human subjects the sugar concentration varied from 67 to 658 mg. per 100 cc. In 80 per cent of the specimens the sugar content exceeded 300 mg. per 100 cc. The Shaffer-Hartmann method was utilized in these analyses. Fresh specimens of semen collected and maintained under aseptic conditions and containing motile spermatozoa showed a decrease in sugar when incubated at 38° C. for 6, 9, 12 and 24 hours. The final concentration of sugar after 24 hours incubation amounted to from 10 to 25 per cent of the original concentration. No glycolysis was noted in specimens placed in a water bath at 100° C. for 5 minutes before incubation. The lactic acid of fresh semen varied from 95 to 137 mg. per 100 cc., determined by the Brehme and Brahdy modification of the Clausen method. During incubation, an increase in lactic acid was noted coincident with the fall in sugar. Lactic acid, however, is not the end product of glycolysis, since in some specimens, the initial increase in lactic acid was followed by a subsequent fall. With Cullen's method the average pH of fresh semen was found to be 7.6. During the first 6 hours of glycolysis the pH rose to 8.0, and subsequently it fell to an acid reaction.

3691

The Effect of Acetyl Thyroxin on the Teeth of Newborn Rats.

MARGARET M. HOSKINS. (Introduced by G. B. Walker.)

From Department of Anatomy, New York University College of Dentistry.

In the acetyl derivatives of thyroxin certain parts of the thyroxin molecule have been replaced by the acetyl group giving a compound which does not affect the metabolic rate of mammals.[1] It retains, however, its effect on the development of young organisms. Tadpoles, to which acetyl thyroxin is given by injections or feeding, develop rapidly into frogs. Newborn rats are similarly affected, going through changes of form normal for the first 25 days of life in a period of 15 days. These general changes are shown in the proportions of the skull, the development of the hair, eyes, auricles and feet. These effects, and the method of the experiments have been described elsewhere.[2] During the course of the work it was noted that the injections hastened the eruption of the incisors, but this point was not studied in detail. A brief sketch of the changes occurring in incisors and molars is presented below.

Animals were injected as in the above experiments, with the same general results. On the third, fifth, and eighth days of life, specimens were killed. In each case a normal litter mate was killed also and studied for comparison. The heads of the animals were fixed in Bouin's fixing fluid. The upper and posterior part of each head was then cut away, leaving the jaws, tongue and surrounding tissues to be sectioned. This piece was decalcified in weak nitric acid, embedded and sectioned.

In material prepared in this way, the following conditions were observed. In a normal rat at 3 days of age, the incisors began to penetrate the oral epithelium, the tips being about a third of the way from the base to the surface. In the injected animal, after only 0.1 mg. of acetyl thyroxin had been given, the tooth had grown considerably farther into the epithelium, its tip being half to three-quarters of the way through. The labial groove is also noticeably more advanced in its development in the injected than in the normal rat. By the fifth day the incisors of injected animals are fully erupted. The surface of the epithelial layer is intact over the tip of the incisors of normal rats at this time. Eruption does not normally occur until the eighth, ninth, or tenth day.

In contrast to the conditions just described in incisors, the molars of newborn rats are not affected by injections of acetyl thyroxin.

In the same specimens which show erupted incisors at 5 days of age, the stage of development of the molars is not to be distinguished from that of the controls; and in older specimens the same lack of effect is to be observed.

It has been thought by some that enamel formation is particularly subject to thyroid influence, and on that account a special study has been made of that process. It is found that enamel formation is undoubtedly accelerated by the treatment. A well defined band of enamel is present in injected rats at 3 days of age, while in the controls the process of its formation has barely begun. If, however, one compares the incisor of a 5-day rat, just at the time of eruption, with that of a normal specimen at 8 days, the same amount of enamel will be found in each. It is evident that although enamel formation is stimulated by acetyl thyroxin, it does not show any greater degree of response than any other process of tooth formation.

It should be noted that animals injected with acetyl thyroxin are not truly hyperthyroid individuals. The over-functioning thyroid accelerates metabolism as well as differentiation, while acetyl thyroxin stimulates the latter process only. Therefore our conclusions concern the rate of development, but not abnormalities of the fully formed tooth due to changes in the metabolic rate of the adult. It is clear that the rate of development of the incisors of rats is influenced by the thyroid. In this respect the teeth are like various other structures of the body; for instance the nasal bone, which grows with abnormal rapidity in these same animals. The development is normal in character, but abnormal in rate.

It should also be remembered that the incisors of rats are different from human teeth and from the molars of rats, in that they grow actively throughout the life of the animal. Attempts have been made to correlate overgrowth of certain teeth in human beings with particular endocrine abnormalities; for instance, enlarged canines have been considered by some to be a sign of excessive adrenal activity. Although it has been shown in the above study that the growth of the incisors of rats is more accelerated than that of the molars, the facts do not warrant the conclusion that human incisors are more influenced by the thyroid than other teeth. It is more probable that the difference observed is due to the peculiarly active growth tendency in rat incisors.

The experiments give no explanation for enamel deficiencies in hypothyroid patients. Amelification is stimulated by the injections, but not more than any other process of tooth development. One must conclude that the fragile enamel seen in hypothyroid patients is a result of disturbed metabolism, not a developmental anomaly.

---

[1] Swingle, W. W., *Am. J. Physiol.*, 1924, lxx, 70.

[2] Hoskins, M. M., *J. Exp. Zool.*, 1927, iv, 48.

3692

Pressor Effect of Guanidine Salts on the Non-anesthetized Rabbit.

R. DOMINGUEZ. (Introduced by J. M. Rogoff.)

From the H. K. Cushing Laboratory of Experimental Medicine, Western Reserve University.

A possible relationship between guanidine intoxication and essential hypertension has been suggested.[1] It was of interest to study the question in non-anesthetized animals. This report contains the results of daily examinations for blood pressure and pulse rate in 12 experiments performed on 5 rabbits provided with a good carotid loop (Van Leersum). The salts used (Eastman Kodak Co.) were methylguanidine nitrate (9 experiments), methylguanidine sulphate (2 experiments), and guanidine nitrate (1 experiment). All the rabbits received 0.1 gm. per kilo of body weight but in different concentrations, 1:10 and 1:20 in distilled water, and 1:20, 1:30 and 1:50 in salt solution (4 and 8 per 1000). The total volume of fluid injected was from 3.7 to 8.2 cc. of distilled water and from 6 to 18 cc. of saline. The weights of the rabbits varied from 2.945 to

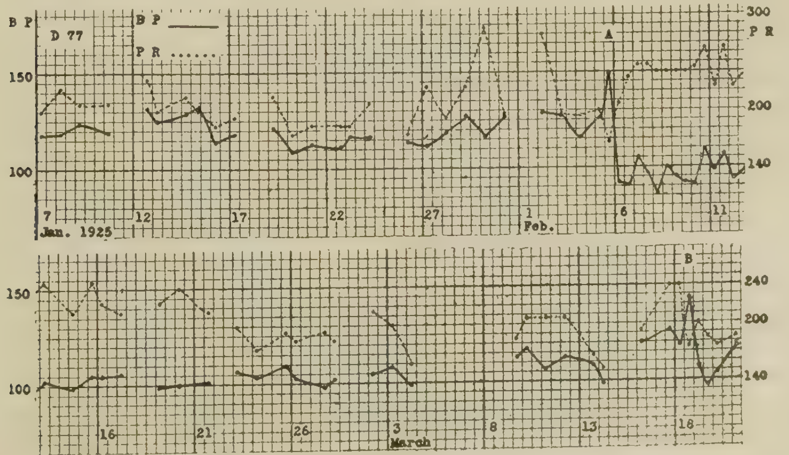


Figure 1.

4.125 kg. After several weeks of observation, the animals received one of the guanidine salts, injected slowly intravenously (marginal vein of the ear). Blood pressure, pulse rate and general reactions were carefully observed immediately after the injection and daily thereafter.

The behavior of blood pressure and pulse rate, in one of the animals, is shown in Fig. 1. Blood pressure is expressed in mm. Hg. (scale to the left); pulse rate in beats per minute (scale to the right). The space between two vertical lines corresponds to one half day. Blood pressure values are the mean of 10 consecutive readings. After the first injection, A, the blood pressure fell and the pulse rate increased, in marked contrast to their behavior before the injection. This condition lasted 18 days after the injection. At B, another injection of same amount of same salt (8.4 cc. of 1:20 in 4:1000 saline) had a similar effect immediately following the injection, but practically no after effect. The graph after this point is similar to the portion before the injections, and has been omitted. The graph under B is a good example of most of the experiments. In a few instances the dip of the pulse rate is more pronounced, reaching 128 per minute, in a few others the peak of the blood pressure is higher.

In one animal the effect after injection was more pronounced than shown in A. The blood pressure oscillated about 80, the pulse rate about 300. The animal died on the fifth day. At autopsy a marked dilatation of the heart was found, hydrothorax (bilateral) and ascites. There is little doubt that methylguanidine produced in this rabbit a severe injury of the myocardium.

The observations may be summarized thus:

1. In no instance did blood pressure reach 170 mm. Hg., *i. e.*, within normal limits.[2, 3] The highest reading was 165 mm. The highest averages were: 150-159 mm. Hg, 2 instances; 140-149, 7; 130-139, 0; 120-129, 3. The last three are negative as far as blood pressure is concerned. Two of these negative results (in the same animal) show that with this dose the pressor effect of methylguanidine may be absent. The other occurred with guanidine nitrate. De Waele and Bulcke have already said that: "Chez le lapin, même à hautes doses, la guanidine reste sans effet sur la pression sanguine et sur le vague."[4]

2. The pulse was slowed in all cases even when the blood pressure was not greatly altered. Slowing of the pulse rate is, in these experiments, more conspicuous than in Alles's report.[5] My results with methylguanidine salts are comparable to his, and, in a few instances more pronounced than the effect he obtained with ethylguanidine.

The difference may be accounted for by the urethane anesthesia and the smaller doses of methylguanidine used by him. Practically all workers agree that this circulatory effect of guanidine salts is independent of the vagus.[1, 4, 5]

3. The time relation of blood pressure and pulse rate effects cannot be made as precise with the method I have used as with a continuously recording device. I find that the highest blood pressure and the lowest pulse rate coincided in 8 out of 12 experiments and that in the remaining 4 experiments the pulse rate effect preceded the rise in blood pressure. The slowest pulse rate was recorded as early as 5 minutes and as late as 49 minutes after the injection. The rise in blood pressure was recorded as early as 8 minutes and as late as 2 hours and 10 minutes after the injection. The blood pressure curves had a single peak in 7 experiments, and a plateau in 5; this plateau was observed to extend for from 35 to 272 minutes. The pulse rate curve had an inverted peak in 8 experiments; and a low flat part in 4, the latter extending in an interval of time varying from 26 to 95 minutes. The blood pressure rise lasted more than the slowing of the pulse in 5 instances, about the same in 4, and less in 3.

4. The other signs of intoxication occurred as follows: salivation (11 instances out of 12 experiments), jerks of the extensors of neck (8 instances), diarrhea—soft feces (7), clonic spasms of legs (6), dyspnea, occasionally violent (4), tremor of lips (3). Of these signs, salivation was first to appear and diarrhea the last to disappear.

5. In a subsequent experiment, part of the graph being reproduced, the injection was repeated when the blood pressure rise of the first injection was beginning to subside. Immediately after the second injection the animal became very weak, the blood pressure rose again (maximum 165 mm. Hg.) without any slowing of the pulse rate, and the respiratory movements became very frequent. Half an hour later the blood pressure was 132 mm. Hg., pulse rate 284 per minute, and dyspnea violent. Eighty minutes after the second injection the animal went abruptly into collapse. The pulse was imperceptible, respiratory movements progressively weaker. The animal died 5 minutes later.

6. The circulatory effect of the methylguanidine salts used is inconspicuous when compared with the general picture of the intoxication.

7. One animal died with all the signs of circulatory embarrassment due to myocardial weakness, as shown at the autopsy by dilatation of the heart, hydrothorax and ascites.

8. A toxic theory of essential hypertension would be more plausible if a substance were found which causes, in normal animals, persistent elevation of blood pressure above normal limits, without obvious signs of general intoxication.

---

[1] Major, R. H., and Stephenson, W., *Bull. Johns Hopkins Hospital*, 1924, xxxv, 186, 140.

[2] Dominguez, R., *J. Met. Res.*, 1924, vi, 123.

[3] Dominguez, R., *J. Exp. Med.*, 1927, xlvi, 443.

[4] De Waele, H., and Bulcke, G., *Arch. int. Physiol.*, 1925, xxv, 74.

[5] Alles, G. A., *J. Pharmacol. Exp. Therap.*, 1926, xxviii, 251.

3693

An Improved Arrangement for Bacteria-Retaining Filters.

STUART MUDD.

From the Henry Phipps Institute, Philadelphia, Pa.

Attention has lately been redirected to the importance of *adsorption* in filtration through bacterial filters[1]; however, if filtration is prolonged, the adsorbing surface may become saturated and substances at first retained may appear in the filtrate. The range of particle size from the smaller filterable viruses to the ordinary bacteria, on the other hand, is a critical zone in which *mechanical retention* plays an increasingly important and finally a determinative rôle.[2] Certain ultramicroscopic viruses may pass the more porous filters in almost undiminished concentration, others in greatly reduced concentration. The visible microorganisms pass the filters only in sufficient numbers to inoculate the filtrate, and only pass at all when certain attributes of the microorganism (*e. g.*, small size, motility, flexibility), or the circumstances of the filtration are especially favorable.

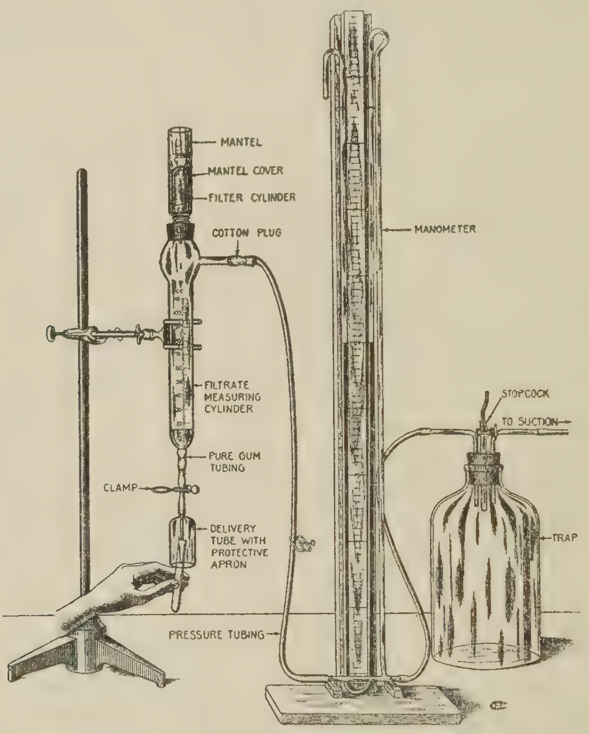
It does not suffice, then, to report that a certain virus is or is not filterable through certain filters.[3] The circumstances must also be known. Incomplete recording of the details of procedure, together with diversity of filtration methods and lack of exact experimental control of the filtration technique, have often introduced needless confusion into the literature on filterable viruses.

The arrangement shown in the accompanying figure offers advantages in point of control over the filtration process, as well as in convenience of operation. The reduced filtration pressure is shown by the manometer. The filtrate is received into a graduated vessel.

TABLE.

Suction approximate time		Manometer reading, approximate	Pressure, mm.Hg., approximate	Volume filtered	Tube inoculated	Inoculum	Results		
On	Off						After 18 hrs.	After 24 hrs.	After 5 days
3:58	3:59	480-265	215	18 cc.	No. 1	8 cc.	Clear	Clear	Clear
					No. 2	5 cc.	Clear	Clear	Clear
4:01	4:02	485-268	217	20 cc.	No. 3	5 cc.	Clear	Clear	Clear
						5 cc. discarded			
					No. 4	10 cc.	Clear	Slight clouding	Dense clouding
4:04	4:05	490-265	225	20 cc.	No. 5	5 cc.	Clear	Slight clouding	Dense clouding
						5 cc. discarded			
					No. 6	10 cc.	Clear	Dense clouding	Dense clouding
4:07	4:14	490-265	225	30 cc.	No. 7	7 cc.	Clouded	Dense clouding	Dense clouding
		465-300	165			10 cc. discarded			
					No. 8	10 cc.	Clouded	Dense clouding	Dense clouding
4:16	4:22	490-265	225	20 cc.	No. 9	5 cc.	Clouded	Dense clouding	Dense clouding
					No. 10	5 cc.	Clouded	Dense clouding	Dense clouding
					No. 11 (control)	5 cc. filtrate and loop-full of unfiltered suspension	Clouded	Dense clouding	Dense clouding

Filtration may be interrupted at any moment by stopping the suction pump and opening the stop-cock on the trap to equalize pressure. Measured volumes of filtrate are then inoculated sterilly into any desired number of culture tubes through the delivery tube with protective apron. The pinch-clamp is released, the stop-cock closed, and suction is continued until another volume of filtrate is collected, and so on indefinitely. A protocol will illustrate:



Filtration of *V. percolans,* 14 day culture in plain broth. Filter, Berkefeld V, 2½ x 5/8 in.; original air-test, 287 mm. Hg.; filter had been used once previously for bacterial filtration, washed from within out with tap-water and resterilized in autoclave. Culture diluted with Ringer-Locke solution and filtered through cotton, then through Berkefeld. Filtrate inoculated into tubes containing plain broth, I-VI-27. Results in table. Darkfield examination shows typical *V. percolans* in clouded tubes.

Thus the first volume of filtrate remained sterile, the succeeding portions showed inoculation in increasing amounts.

The basic arrangement of which this is a modification has been used for some years by J. D. Aronson for sterilizing and tubing immune sera. His small filtrate-receiving cylinders without calibration are less expensive. The so-called mantel cover, an inverted tube over the filter candle, enables all but the last traces of liquid to be filtered; this device was introduced years ago by M. P. Ravenel. Cylinders of both types, delivery tubes and mantle covers are stocked by Arthur H. Thomas Company of Philadelphia.

---

[1] Kramer, S. P., *Science, N. S.*, 1927, lxv, 45; *J. Infect. Dis.*, 1927, xl, 343.

[2] Mudd, S., in T. M. River's, Filterable Viruses, in press.

[3] *Cf.* Krock, F. H., and Holman, W. L., *J. Am. Med. Assn.*, 1923, lxxx, 1142.

3694

Vitamins A and B in the Chinese Litchi Nut.

ARTHUR H. SMITH AND PETER P. T. SAH.

From the Laboratory of Physiological Chemistry, Yale University.

The Chinese hazel or litchi nut *(Litchi Chinensis)* has long been cultivated for medicinal and edible purposes in the Orient, and has attained a certain vogue in this country. A study of the therapeutic and food values of this fruit was made by Read, who describes the nut as "nearly globose with a dull brick-red pericarp which, when ruptured, exposes a sweet, brown, fleshy arillus surrounding a glossy chestnut-brown obicular seed . . . ."[1] If this fleshy edible part corresponds in nutritive value to most of the fruits used in the American dietary, it should constitute a source of certain of the accessory food factors. The present study deals with the assay for vitamins A and B in the litchi nut.

Young rats 25 to 30 days old, weighing 40 to 50 grams, were given the following diets:

Lacking vitamin A		*Lacking vitamin B*	
Extracted casein	18%	Extracted meat residue	18%
Raw corn starch	50	Raw corn starch	50
Crisco	28	Crisco	23
Salt mixture[2]	4	Cod liver oil	5
Dried yeast 200 to 400 milligrams daily		Salt mixture[2]	4

The growth of rats on the first ration was rather prolonged, lasting from 40 to 50 days before cessation. The diet was then irradiated with light from a quartz mercury vapor lamp, with the result that growth was immediately resumed for 10 to 14 days more. At that time definite ophthalmia had developed in all the animals, and the edible part of the litchi nut was given in doses of 1 and 2 grams daily (0.87 to 1.74 grams dry matter).* The material was not always eaten completely, and the food consumption was irregular during this period. In no case was the ophthalmia cured, nor did the body weight increase. When 6 to 10 drops of cod liver oil were given with 2 grams of litchi nut, growth was secured, and the ophthalmia disappeared in 5 to 8 days.

In the test for vitamin B, the rats, after consuming the basal ration for approximately 2 weeks, ceased growing, and litchi nut was given in doses of 1 and 2 grams daily. There was not the slightest effect in checking the loss of weight, although the material was well consumed. In view of the usual prompt though temporary response of rats to even traces of vitamin B under these conditions, the observed behavior of the experimental animals in the present tests indicates that the litchi nut contains little, if any, vitamin B. When 200 milligrams of dried yeast were given with 2 grams of litchi nut, growth at the normal rate was resumed.

It is thus apparent that, while the fruit contains ineffective quantities of vitamins A and B, it is not toxic as has been stated in the Chinese Materia Medica (See also Read[1]). On the other hand, the dried litchi nut as obtained on the American market and as used in these experiments, constitutes an exception among edible fruits in its lack of the two food factors, especially vitamin B.

---

* The edible portion showed 12.6% loss in weight when heated for 120 hours, at 60° C., in a vacuum oven.

[1] Read, B. E., *J. Am. Chem. Soc.*, 1918, xl, 817.

[2] Osborne, T. B., and Mendel, L. B., *J. Biol. Chem.*, 1918, xxxvii, 557.

3695

Variations in Auriculoventricular Conduction Time in Rheumatic Carditis with Salicylate Therapy.

ROBERT L. LEVY AND KENNETH B. TURNER.

From the Department of Medicine of the College of Physicians and Surgeons, Columbia University, and the Presbyterian Hospital, New York City.

It is now generally believed that the drugs employed in the treatment of acute rheumatic fever (sodium salicylate, acetylsalicylic acid and neocincophen) probably exert an antisymptomatic rather than a specific action in this disease. Following their administration, the pain and exudation of arthritis are usually controlled, high fever is reduced, the heart rate becomes slower and there is often a concomitant fall in the leucocyte count. The mechanism by means of which these results are accomplished, is not understood.

No direct effect of medication upon the cardiac lesions of rheumatic fever has been demonstrated. Auscultatory signs do not afford an accurate index of slight modification in a valvulitis. Electrocardiographic evidence of myocardial involvement is frequent; and the most readily measurable graphic change is auriculoventricular conduction time (P-R interval).

In three cases of acute rheumatic carditis with arthritis, prolonged P-R interval (0.23-0.24 seconds) was found on admission to the hospital. No digitalis had been given to any of the patients. Fever, tachycardia and slight leucocytosis were present in all. Salicylate, in the form of sodium salicylate or aspirin, was administered in courses, with an interval of from six to nine days between them. During this interval all drugs were withheld. Following the institution of salicylate therapy, the usual effects on arthritis, temperature, pulse and leucocyte count were experienced. In addition, there was a gradual reduction in the duration of the P-R interval to within normal limits (0.20 seconds or less). On discontinuing salicylate, the conduction time increased beyond the upper limit of normal, in one instance becoming longer than on first examination. The reappearance of fever, tachycardia, leucocytosis, and in two instances, arthritis, afforded further evidence of the recrudescence of acute infection. A second course of salicylate was again followed by the subsidence of these signs and by a shortening of A-V conduction. There appeared to be a definite relationship between variations in conduction time and salicylate medication.

In a number of cases of partial heart block occurring in the course of rheumatic fever, post-mortem examination of the A-V bundle has disclosed characteristic inflammatory lesions involving the fibres of this structure. It has been emphasized by Swift[1] that it is predominantly the exudative manifestations of rheumatism which are modified by antirheumatic drugs. In the cases here described, it is conceivable that salicylate caused diminution of edematous infiltration in the region of the bundle, with resultant shortening of conduction time.

These observations suggest the conclusion that in certain cases of acute rheumatic fever with myocardial involvement, as evidenced by prolongation of A-V conduction time, administration of salicylate exerts a favorable effect upon the lesions in the heart muscle.

---

[1] Swift, H. F., *Am. J. Med. Sci.*, 1925, clxx, 631.

Western New York Branch.

Cornell University, October 15, 1927.

3696

New Conception of the Mechanism of Epinephrine Glycosuria.

CARL F. CORI AND GERTY T. CORI.

From the State Institute for the Study of Malignant Disease, Buffalo.

It has been shown previously[1] that liver glycogen is mobilized in fasting adrenalectomized animals when they receive insulin, or, is formed less rapidly, when they receive glucose plus insulin. This result led to an investigation of the influence of small doses of epinephrine on the carbohydrate metabolism of normal rats. During glucose absorption, epinephrine in doses of 0.02 mg. per 100 gm. rat, produced the following effects, with great regularity. It increased heat production, decreased the rate of intestinal absorption, and caused glycosuria. On an average, 6% of the glucose absorbed during 4 hours was excreted in the urine. An equally constant result was increased glycogen formation in the liver, demonstrable not only after 4 hours, but also after 1, 2 and 3 hours of glucose absorption. For the 4 hour period a complete balance of the absorbed glucose has been made. A comparison with the balance sheet of control rats shows:

Per 100 parts of absorbed glucose, x parts are:

	oxidized	deposited as liver glycogen	deposited as body glycogen	excreted in urine
Glucose alone	44	18	25	0
Glucose plus epinephrine	39	23	15	6
Difference	—5	+5	—10	+6

It will be noted that glycogen formation in the muscles and glucose oxidation are diminished, while glycogen deposition in the liver is increased. This indicates that the muscles are unable to dispose of part of the absorbed glucose. According to our present conception, the sugar which the muscles fail to utilize, which is therefore excreted in the urine, is derived from liver glycogen. A part of the glycogen deposited in the liver during glucose absorption is mobilized by epinephrine. This sugar cannot be utilized by the muscles, for an unknown reason, while the glucose which reaches the muscle directly from the intestine is utilized in a normal way.

Experiments with the same dose of epinephrine have also been made on rats in the post-absorptive state. As much glucose was fed by stomach tube as is absorbed in 4 hours, and the metabolism was determined during the 4 hours. Since the amount of glucose absorbed and oxidized is known, the amount of glycogen formed in liver and muscles during the 4 hours can be calculated. After the 4 hours of glucose absorption, epinephrine or insulin was injected, while control animals were given an equivalent amount of saline. The metabolism was determined for 3 hours after the injection. Then the animals were killed, and glycogen in the liver, and in the rest of the body was determined.

The following table indicates how much glycogen disappears from liver and muscles during 3 hours of fasting, and how much glucose is oxidized during that time. If the calculated loss of glycogen is correct, it should check with the observed oxidation of glucose. It will be noted that the agreement between these two values is satisfactory. This justifies the use of the method outlined above.

TABLE I.

Disappearance of liver and muscle glycogen (in mg. of glucose) after epinephrine and insulin injections. The values are per 100 gm. of body weight per 3 hours. Each figure is an average of 4 experiments.

	Liver glycogen	Muscle glycogen	Glucose in body fluids	Glucose disappeared	Glucose oxidized	Blood sugar
Control	—53	—171	—23	**247**	**221**	113
Epinephrine	+14	—312	+5	**293**	**271**	174
Insulin	—143	—191	—44	**378**	**434**	69

The same dose of epinephrine which produced glycosuria during active glucose absorption, caused only hyperglycemia when the animals were in a post-absorptive state. There was an increase in heat production, which was met in part by an increase in carbohydrate oxidation. It will be noted that normal animals oxidized 221 mg. of glucose, while those receiving epinephrine oxidized 271 mg. Where did the glucose which was oxidized come from? In the normal animal about ¼ came from the liver and about ¾ from the muscle glycogen. In the animals with epinephrine none came from the liver, indicating that the sugar mobilized in the liver is not utilized by the tissues, and is therefore reconverted in part into liver glycogen. All the carbohydrates that the epinephrine animals oxidized came from muscle glycogen. In the insulinized animals there was a strong increase in carbohydrate oxidation, with approximately an equal disappearance of liver and muscle glycogen. Contrary to expectation, it is not epinephrine but insulin which causes a disappearance of liver glycogen in fasting rats.

The generally accepted assumption is that epinephrine causes glycosuria because it mobilizes liver glycogen. Recent experiments on fasting rabbits make it certain that mobilization of liver glycogen alone cannot account for the epinephrine glycosuria. It was argued that if mobilization of liver glycogen is the only factor, the problem is obviously one of glucose tolerance. It should make no difference whether glucose is injected into a peripheral vein, or whether the sugar is given off by the liver into the *vena cava*. The tolerance limit of rabbits for intravenously injected glucose is at a rate of 0.9 gm. per kilo per hour.

If epinephrine glycosuria is caused by mobilization of liver glycogen only, the rate at which sugar is given off by the liver must be greater than 0.9 gm. per kilo per hour. Such rates of glycogen mobilization have not been found during epinephrine glycosuria. With rates as low as 0.2 gm. per kilo per hour, more than 50% of the glucose given off by the liver were recovered in the urine, while about 35% were retained in the blood and body fluids. This indicates that only a negligible quantity of the sugar given off by the liver following epinephrine injections is utilized by the tissues. However, it must not be supposed that this lack of utilization is of the nature of a diabetic disturbance, because, as the experiments on rats have shown, glucose reaching the muscles directly from the intestine, or glycogen already present in the muscles, is utilized in a normal way. It is the sugar circulating in the blood, originally derived from liver glycogen, which the tissues are unable to attack for a reason as yet unknown. Consequently, a part of this sugar is excreted in the urine, while another part may be reconverted into liver glycogen. Reconversion into liver glycogen is also suggested by the experiments of Pollak[2] and Kuriyama,[3] who found storage of liver glycogen after daily injections of epinephrine into fasting rabbits. The full implication of our results will be given in more detail in later papers.

---

[1] Cori, C. F., and Cori, G. T., *J. Biol. Chem.*, 1927, lxxiv, 473.

[2] Pollak, L., *Arch. exp. Path. u. Pharm.*, 1909, lxi, 166.

[3] Kuriyama, S., *J. Biol. Chem.*, 1918, xxxiv, 269.

3697

A Substance Which Prolongs the Life of Adrenalectomized Cats.

F. A. HARTMAN, C. G. MAC ARTHUR AND W. E. HARTMAN.

From the Physiology Laboratory of the University of Buffalo.

We have obtained a substance from the cortex of the adrenal (ox), which prolongs the life of adrenalectomized cats.

The water or 0.8% NaCl extract is cleared of much protein and cellular debris by isoelectric precipitation. The substance is then obtained by precipitation with NaCl. Epinephrin is removed by washing.

At present we have used this preparation in the treatment of 21 adrenalectomized cats. Two of these cats are not included in this report, because one died from being overheated, and the other is still alive after 175 days, no injections having been given for 100 days.

The average length of life of untreated adrenalectomized cats may be taken as 5 to 6 days, judging from the work of Marine and Baumann,[1] and our own unpublished results.

The first 5 cats in this study lived an average of 15.6 days, although we were constantly modifying our preparation, and at times there was evidence of deterioration. The results were complicated, or the experiment even terminated in some, by infection (Table I).

TABLE I.

Cat	Survival	Remarks
	days	
BG	17	
BH	8	Respiratory and skin infection
BI	18	Died from pneumonia
BK	18	Died from empyaemia
BO	17	Thyroids removed 17 months before

Average 15.6.

In the remainder of the experiments the methods of preparing the material for injection have been more uniform. Likewise, the survival periods are longer. In Table II we have included experiments which may have been shortened by infections or reduction in kidney tissue.

In Table III there were no complications except in BW. In this cat death seemed to be hastened by the feeding of too much dextrose. Up to this time the cat had been in excellent condition and had eaten well.

TABLE II.

Cat	Survival	Remarks
	days	
BM	12	Piece of kidney removed one week before last operation
BY	7	Died of acute respiratory infection
BZ	23	Middle ear full of pus
CB	10	Bad skin infection
CC	21	Undrained pus pocket
CD	22	Lungs consolidated
CE	27	Gangrene
CH	21	Lungs consolidated

Average 17.9.

TABLE III.

Cat	Survival	Cat	Survival
	days		days
BR	60	CA	11
BW	28	CF	31
BX	40	CG	14

Average 30.7.

The average survival of the 19 cats was 21 days.

Many of these cats behaved like normal cats, eating well, showing spontaneity and taking an active interest in their surroundings. Manipulations, such as drawing daily samples of blood, or even more drastic procedures, produced no ill effects. The reactions and behavior were a decided contrast to untreated adrenalectomized animals.

The amount of fluid injected was so small that it could not have been a factor in prolonging life or preventing the onset of symptoms.

The essential hormone of the adrenal cortex must have been present in our preparation. Otherwise it is impossible to account for our results.

We propose Cortin as the name for this hormone.

---

[1] Marine, D., and Baumann, E. J., *Am. J. Physiol.*, 1927, lxxxi, 99.

3698

The Effect of a Meal upon the Titratable Alkalinity of Blood.

ROGER S. HUBBARD.

From the Clifton Springs Sanitarium and Clinic, Clifton Springs.

The effect of a meal upon the titratable alkalinity of the blood was studied in 47 subjects by the method of Sumner and Hubbard.[1] Specimen's were obtained after a night's fast and two hours after a meal by which the carbohydate tolerance was tested.[2] The meal consists of 100 gm. carbohydrate, 24 gm. protein, and 25 gm. of fat, fed as oatmeal, milk, sugar, dates, bread, butter, orange, and an egg. The subjects were patients in the sanitarium, in whom it was thought that some abnormality of carbohydrate metabolism might be present. The average blood sugar content, determined by Benedict's copper method,[3] was 93 mg. per 100 cc. before, and 115 mg. after the meal was fed. Of the cases studied 14 showed increases in blood sugar after the meal, greater than 25 mg. per 100 cc.; 5 of these were greater than 100 mg. per 100 cc.

The average values obtained before the meal were 44.7 cc. N/10 alkali per 100 cc. of blood when methyl red was used as an indicator, and 28.4 cc. when thymolphthalein was used. After the meal these values were 46.0 and 30.9 respectively. The distribution of the increases and decreases by the two methods is given in the table. In 6 cases the variation in the titration values by the two methods were of opposite sign, but in 4 of these, one of the methods showed variations of 1.0 cc. N/10 alkali or less. There were 2 cases in which no variation in one titration value was accompanied by a more or less significant variation in the other one. In the remaining 39 cases both titrations showed differences which were parallel.

It is evident from the average values and from the table that the majority of the cases showed an increased amount of titratable alkali after the meal with both indicators, but that the average difference was slight. Variations were more pronounced when thymolphthalein was used as an indicator. It seems probable that these differences represent part of the process of the adjustment of the organism to the secretion of hydrochloric acid by the stomach. Two hours after the ingestion of such the meal, gastric digestion is well advanced, and absorption from the intestine progressing actively, so that marked increases in titratable alkali should not be expected. Besides this, variations in gastric secretion are so great that marked differences in the response of any blood factor would almost certainly occur in such experiments.

TABLE I.

Indicator	cc. N/10 alkali per 100 cc. blood.						
	over −5 no.	to −5 no.	to −2 no.	−1 to +1 no.	to +2 no.	to +5 no.	over +5 no.
Methyl red	1	7	2	15	3	15	4
Thymolphthalein	3	4	5	8	2	9	16

Negative values have been used where the concentration of alkali was lower in the specimen collected after the meal than it was in the first one.

The titration to the thymolphthalein endpoint is distinctly less accurate than is that to methyl red, but the author feels that in such experiments as are reported here, where the two analyses are run almost simultaneously under comparable conditions, a fair degree of confidence can be placed in the results. Whether the figures obtained at the more strongly alkaline reaction should be regarded as more than simply confirmatory of those obtained with methyl red, is a point which should probably be regarded as open to question. The decrease in difference between the two values implies a decrease in the soluble acid buffers active in the blood, but whether this really occurs, and represents a part of the accommodation of the organism, cannot be considered as definitely established by the figures given.

---

[1] Sumner, J. B., and Hubbard, R. S., *J. Biol. Chem.*, 1923, lvi, 701.

[2] Brill, I. C., *J. Lab. Clin. Med.*, 1925, viii, 727.

[3] Benedict, S. R., *J. Biol. Chem.*, 1925, civ, 207.

3699

Chemical Analysis of Incubated Non-Fertile Eggs.

GEORGE W. PUCHER.

From the Department of Biochemistry, University of Buffalo Medical School, and the Department of Laboratories, Buffalo General Hospital.

Fresh eggs, procured from White Leghorn chickens, were analyzed to obtain data on the chemical changes occurring before incubation. Then eggs from the same source were incubated, and the non-fertile ones analyzed at various periods of time, up to 20 days, for carbohydrate, and non-protein nitrogen constituents, using a protein free filtrate prepared by the Folin-Wu method. Table I presents a summary of the data obtained.

TABLE I.
CHEMISTRY OF INCUBATED, NON-FERTILE EGGS
(All eggs tested for sterility before analysis.)

A. ALBUMIN.
Mg. per 100 grams.

Substance Analyzed	0 days	5 days	10 days	20 days
	%	%	%	%
Water	88.21	87.3	86.5	85.3
N. P. N.	7.34	8.97	7.12	13.80
Urea N.	1.92	1.10	0.67	3.17
Uric Acid N.	0.20	0.28	0.23	0.23
Amino Acid N (free)	3.28	4.02	5.34	6.41
Free Sugar	464.	466.	441.	432.
Hydrol. Sugar Folin	221.	172.	176.	154.
Hydrol. Sugar Benedict	405.	359.	366.	356.
B. YOLK.				
	%	%	%	%
Water	50.21	49.1	53.6	60.5
N. P. N.	66.7	72.2	64.0	64.7
Urea N.	6.49	5.66	5.52	8.86
Uric Acid N.	0.196	0.15	0.17	0.19
Amino Acid N (free)	49.2	43.3	45.2	47.4
Free Sugar	245.	255.	240.	277.
Hydrol. Sugar Folin	118.	82.	116.	125.
Hydrol. Sugar Benedict	297.	332.	294.	299.

The following conclusions may be drawn:

All (99.2%) of the free sugar of the albumin is fermentable in contrast to the yolk, which contains a small amount of non-fermentable sugar (from 7 to 12%).

The hydrolyzable sugar content of the albumin is greater than that of the yolk. Only a relatively small proportion (40 to 50%) of this is fermentable by yeast.

The free amino acid and non-protein nitrogen content of the yolk is very high, as compared with that of the albumin.

Incubated, sterile, non-fertile eggs do not undergo any appreciable changes in their free sugar, hydrolyzable sugar or non-protein nitrogen constituents, over an incubation period of 20 days.

Holmes Condict Jackson

The Society for Experimental Biology and Medicine records with the most sincere regret the death of Professor Holmes Condict Jackson, which occurred on October the 25th, 1927.

Professor Jackson was one of the group of seven who met with Dr. Meltzer on January 19, 1903, to consider the matter of starting the Society, and with its permanent organization of February 25, 1903, he became a charter member. In 1913 he was elected secretary of the Society, a position carrying with it the editorship of the Proceedings. He held this position for a period of ten years. In 1923 he was elected President, and served in this office for two terms.

Dr. Jackson's interest in the Society was maintained to the end. He was a constant attendant of its meetings and contributed frequently to its programs and discussions. He filled the offices to which he was elected with marked executive ability, and contributed his full share in making the Society a success. Through his death the Society has lost one of its most valuable workers, and the many members who came in close contact with him a personal and dependable friend.

Therefore be it hereby resolved that this statement of Professor Jackson's service to the Society, and the expression of its deep sorrow at his untimely death be recorded on the minutes of the Society.